Skin Cancers in Renal Transplant Recipients: Epidemiology and Pathogenesis

Cristina Bordea

Jesus College

Trinity Term 2001

Nuffield Department of Surgery

John Radcliffe Hospital

Oxford

Thesis submitted in fulfillment of the requirements for the degree of Doctor of Phylosophy at the University of Oxford

RADCLIFFE SCIENCE LIBRARY OXFORD

23 JUN 2003

With grateful thanks to my teachers

and to my mother, Elena

Abstract

Cristina Bordea

Jesus College, Oxford

Doctor of Philosophy

Trinity Term 2001

This thesis investigates the incidence and epidemiological characteristics of skin cancers that have occurred in the Oxford Renal Transplant Centre over a period of 21 years, immune responses and cells associated with the immune response in immunosuppressed patients with and without skin cancer, non-immunosuppressed patients with skin cancers and matched controls, and the effect of HLA and TNF-α polymorphisms on the risk of subsequent skin cancer after transplantation.

The epidemiological study showed a higher incidence and risk of skin cancer after transplantation than previously reported from centres in Europe. The increase is highest for squamous cell carcinoma, the most prevalent tumour type. A detailed anatomical distribution for individual tumour type and gender is provided and risk factors and cumulative incidences are assessed. A possible association with the arterio-venous fistula side is not confirmed. Delayed type hypersensitivity (DTH) responses and non-specific inflammatory responses are investigated in a novel design study. The results suggest reduced DTH responses in renal transplant patients with skin cancer with comparable inflammatory responses. It also confrimed the increased skin dryness in renal transplant patients, possibly a side effect of their medication. Furthermore the number of Langerhans cells and morphology are assessed in the same groups of patients. Transplant patients appear to have Langerhans cells with fewer and shorter dendrites. However the number of Langerhans cells does not appear to be a good predictor of the risk of skin cancer.

The association of genes of the MHC region and polymorphic alleles of the TNF-α gene with therisk of skin cancer after transplantation was studied. An association with HLA B27 and the risk of basal cell carcinoma was found, but no association with the TNF-α alleles investigated.

Acknowledgements

I would like to express my gratitude to my supervisors Professor Sir Peter J Morris and Professor Fenella Wojnarowska. They have both encouraged and supported me and have been tremendous role models. Special thanks are due to Fenella, Muriel and Freddie for their welcoming hospitality and friendship. I am also grateful to Professor Justin Roake who supervised my work between 1996-1997.

I wish to acknowledge the contribution of Janice Cameron from the Department of Dermatology who helped with patient recruiting and immunological testing. I am grateful to Jill Allen and Diane Dean who welcomed me in the Dermatology Laboratory and willingly took on the task of teaching me immunohistochemistry. My thanks are also due to Professor Peter Friedman from the Dermatopharmacology Unit in Southampton and Dr. Carolyn Willis from the Dermatology Research Unit in Amersham for their advice on skin testing; to Dr. Ken Welsh for his support with the genetic studies and Clare Logan for teaching me the laboratory skills; Dr. Peter Millard and Dr. Kevin Hollowood for their help with histopathology; Dr. Mario Cortina-Borja, Dr. Laura Rodriguez for their advice on statistics; Daniel Marshall for his help and expert knowledge of computer software. I would also like to thank Professor Irene Leigh and Professor Kevin Gatter for their gift of monoclonal antibodies.

I am most grateful to all the patients who took part in the clinical studies and to the staff of the Renal Transplant Centre and Dermatology Department for their help and support.

This work would not have been possible without the knowledge and effort of those who taught me over the last twenty years and my mother's love and support.

The work was funded by an Overseas Graduate Scholarship from Jesus College, Oxford and the Jill Broadis Cancer Fund.

Contents

Abbreviations viii

1 Skin cancers and premalignant lesions in renal transplant recipients: epidemiological, clinical and pathological background 1

1.1 Introduction 1

1.2 Epidemiological background 1

1.2.1 Time to presentation 4

1.2.2 Relative risks 4

1.2.3 Anatomical distribution 5

1.2.4 Clinical course 6

1.2.5 Skin cancers in paediatric renal transplant recipients 7

1.3 Clinical and histological aspects 8

1.3.1 Solar keratoses 8

1.3.2 Bowen's disease 9

1.3.3 Keratoacanthoma 10

1.3.4 Squamous cell carcinoma 11

1.3.5 Basal cell carcinoma 13

1.3.6 Malignant melanoma and dysplastic naevi 15

1.3.7 Merkel cell tumour 16

1.3.8 Sebaceous carcinoma 16

1.3.9 Histological features of skin cancers in transplant patients versus non-immunosuppressed patients 17

1.4 Principles of management 17

2 Skin cancers in renal transplant recipients: pathogenesis 23

2.1 Introduction 23

2.2 Ultraviolet exposure 23

2.3 Immunosuppressive drugs 25

2.4 Human Papilloma Virus 27

2.5 Genetic factors 30

3 Skin cancers in renal transplant recipients: a single-centre, 21-year retrospective analysis 35

3.1 Introduction 35

3.2 Patients and methods 36

3.2.1 Patients 37

3.2.2 Data collection 37

3.2.3 Data analysis 39

3.3 Results 39

3.3.1 Descriptive epidemiology 39

3.3.2 Distribution of skin cancers on the upper limb in relation to the side of the arterio-venous fistula 47

3.3.3 Risk factors analysis 48

3.4 Discussion 51

3.4.1 Descriptive epidemiology 51

3.4.2 Distribution of skin cancers in the upper limb in relation to the side of the arterio-venous fistula 55

3.4.3 Risk factor analysis 56

4 The role of immunological factors in the aetiology of squamous cell carcinoma in renal transplant recipients 59

4.1 Background 59

4.1.1 The skin immune system 59

4.1.2 Immune responses to allergens and irritants 62

4.1.3 Immune responses to skin cancers 64

4.2 Study design: patients and methods 66

4.2.1 Identification of cases and controls 66

4.2.2 Methods 68

4.3 Results 77

4.3.1 DNCB 78

4.3.2 Recall antigens 83

4.3.3 Contact allergens 85

4.3.4 Dithranol 86

4.3.5 Sodium lauryl sulphate 89

4.4 Discussion 92

5 The role of genetic factors in the aetiology of skin cancers in renal transplant recipients: HLA types and TNF-α polymorphisms.....97

5.1 Introduction 97

5.2 Human leukocyte antigens and the risk of skin cancer 97

5.2.1 Background 97

5.2.2 Patients and methods 99

5.2.3 Results 100

5.3 TNF-α polymorphisms and the risk of skin cancer 100

5.3.1 Background 100

5.3.2 Patients and methods 101

5.3.3 Results 103

5.4 Discussion 104

6 Langerhans cells: A quantitative and morphological analysis

6.1 Introduction.....107

6.2 Patients and methods.....108

6.3 Results.....112

6.4 Discussion.....116

7 Conclusions.....119

Appendix A 123

Appendix B 146

Bibliography 152

Glossary 169

Abbreviations

Arg	arginine
ATG	antithymocyte globulin
AUC	area under the curve
Aza	azathioprine
BCC	basal cell carcinoma
B cells	B lymphocytes
Ca	carcinoma
CD	cluster of differentiation
CDK	cyclin-dependent kinase
CI	confidence interval
CTTR	Cincinnati Tumour Transplant Registry
CyA	ciclosporine A
Δ	variation (in a parameter)
DNA	deoxyribonucleic acid
DNCB	dinitrochlorobenzene
ESCD	European Society of Contact Dermatitis
EV	epidermodysplasia verruciformis
F	females
GST	gluthatione S transferase
HLA	human leukocyte antigen
HPV	human papilloma virus
ICAM	intercellular adhesion molecule
IFN	interferon
IL	interleukin
IR	irritant reaction
KA	keratoacanthoma
LC	Langerhans cell
LPS	lipopolysaccharide

M	males
MHC	major histocompatibility complex
MM	malignant melanoma
NA	not applicable/not available
NMSC	nonmelanoma skin cancer
NT	not tested
OR	odds ratio
PBS	phosphate buffered saline
PCR	polymerase chain reaction
Pro	proline
py	patient-years
RTR	renal transplant recipients
SCC	squamous cell carcinoma
SD	standard deviation
SK	solar keratosis
SLS	sodium lauryl sulphate
SW	sterile water
T cells	T lymphocytes
TEWL	transepidermal water loss
T_H1	inflammatory CD4 T cells
T_H2	helper CD4 T cells
TNF	tumour necrosis factor
UV	ultraviolet
WSP	white soft paraffin
w/w	weight/ weight (concentration)
w/v	weight/ volume (concentration)

Chapter 1

Skin Cancers and Premalignant Lesions in Renal Transplant Recipients: Epidemiological, Clinical and Pathological Background

1.1 Introduction

Renal transplantation is an established treatment for patients with end stage renal failure (Morris, 1988). The first indication that immunosuppressed transplant recipients were susceptible to cancer came with the transplantation of apparently normal kidneys removed from patients dying with cancer. It was soon recognised that such organs could harbour malignant cells that could proliferate when transplanted in the immunosuppressed recipient (Martin *et al.*, 1965; McPhaul *et al.*, 1965). A few years later the first reports of cancer arising *de novo* in transplant patients were published in the medical press (Doak *et al.*, 1968; Penn *et al.*, 1969).

1.2 Epidemiological background

In the long term following kidney transplantation, skin cancers represent the most common malignancy in patients of European descent (Bouwes Bavinck *et al.*, 1996; Disney *et al.*, 1997; Gaya *et al.*, 1995; London *et al.*, 1995; Penn, 1994a). The first report of an increased risk of skin cancer in transplant patients was published by Walder et al, in 1971 (Walder *et al.*, 1971).

They described the occurrence of multiple skin cancers in 14% of a group of Australian patients who received a kidney transplant 4-45 months previously and who were treated with azathioprine and prednisolone, none of whom had had skin cancer before transplantation. The clinical features described in this short paper summarise remarkably well the main characteristics of skin cancers developing in this group of patients: reversal of the squamous cell carcinoma/basal cell carcinoma ratio reported in the general population, tendency for the lesions to be multiple, older age at transplantation of the patients who subsequently developed skin cancer, presence of atypical "recurrent keratoacanthomas", increased prevalence of keratoses on sun exposed sites with rapid evolution of some of them into squamous cell carcinomas (Walder *et al.*, 1971).

Since then reports have been published from transplant centres across the world that investigated the incidence and/or prevalence and clinical features of skin cancers in renal transplant recipients. Comparisons between results published from different centres have to take into account differences in the study design: criteria for patient inclusion, length of follow-up after transplantation, type of premalignant and malignant lesions included, time of data collection [retrospective, prospective, cross-sectional]. A summary of published data is given in Table 1.

The cumulative incidence rises with the time after transplantation yet varies with the level of sun exposure. In Queensland, a state of Australia situated at approximately 20° latitude in the Southern hemisphere the cumulative incidence of basal cell carcinoma [BCC] and/or squamous cell carcinoma [SCC] rose from 7% at 1 year to 45% at 11 years and 70% at 20 years after transplantation (Bouwes Bavinck *et al.*, 1996). Comparatively, the cumulative incidence for BCC and/or SCC in the Netherlands, a country situated at approximately 50° latitude North, was 0.2%, 16%, 41% at the same time points (Hartevelt *et al.*, 1990).

The premalignant and malignant skin conditions that have been reported to occur most frequently in transplant recipients include solar keratosis [Fig. 1.4],

keratoacanthoma, Bowen's disease [Fig. 1.6], basal cell carcinoma [Figs 1.12-1.14], squamous cell carcinoma [Figs. 1.2, 1.8, 1.10], malignant melanoma and Kaposi sarcoma. In addition, cases of angiosarcoma [Fig. 1.1], Merkel cell carcinoma (Gooptu *et al.*, 1997; Penn, 1994a), sebaceous carcinoma (Blohme &

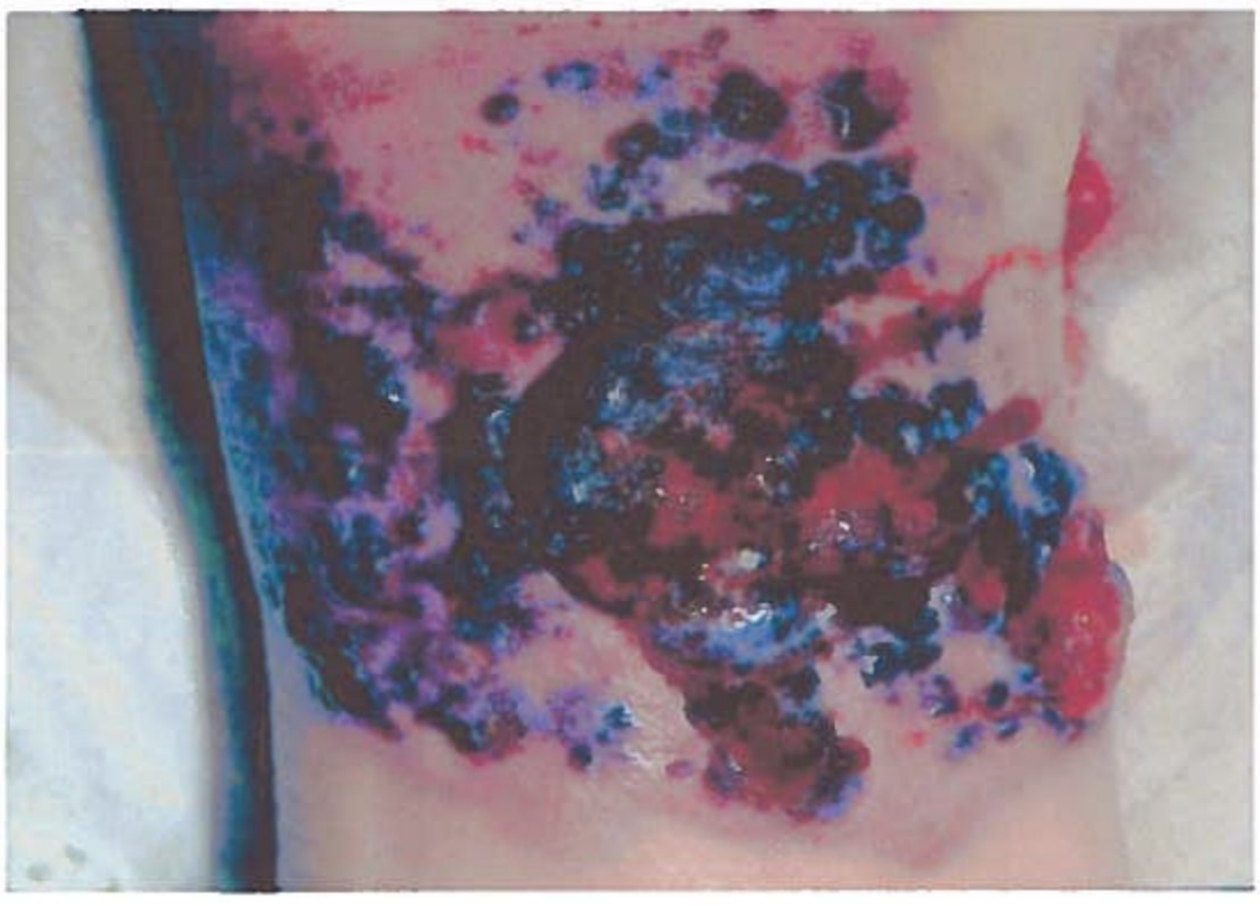


Fig. 1.1 Angiosarcoma in a renal transplant recipient: haemorrhagic nodules over a raised, indurated purple plaque.

Larko, 1990; Penn, 1994a) and a pure cutaneous plasma cell tumour have also been reported (Whittam *et al.*, 1996). Of these, squamous cell carcinoma was the most common in long-term retrospective studies (Liddington *et al.*, 1989; London *et al.*, 1995; Sheil *et al.*, 1992). However in a Hispanic renal transplant population followed-up every 6 months for the first 1-3 years after transplantation, basal cell carcinoma was diagnosed more often than squamous cell carcinoma (Ferrandiz *et al.*, 1995).

In non-immunosuppressed patients basal cell carcinoma is the most common skin tumour. In renal transplant recipients, squamous cell carcinoma outnumbers basal cell carcinoma and the ratio of squamous cell carcinoma/basal cell carcinoma reverses compared with the general population: from 1:4 to 1.5:1 in Australia (Bouwes Bavinck *et al.*, 1996) and from 1:8 to 3.6:1 in the Netherlands (Hartevelt *et al.*, 1990). It is interesting to note that the reversal is less pronounced in Australia and New Zealand than in Europe [Table 1].

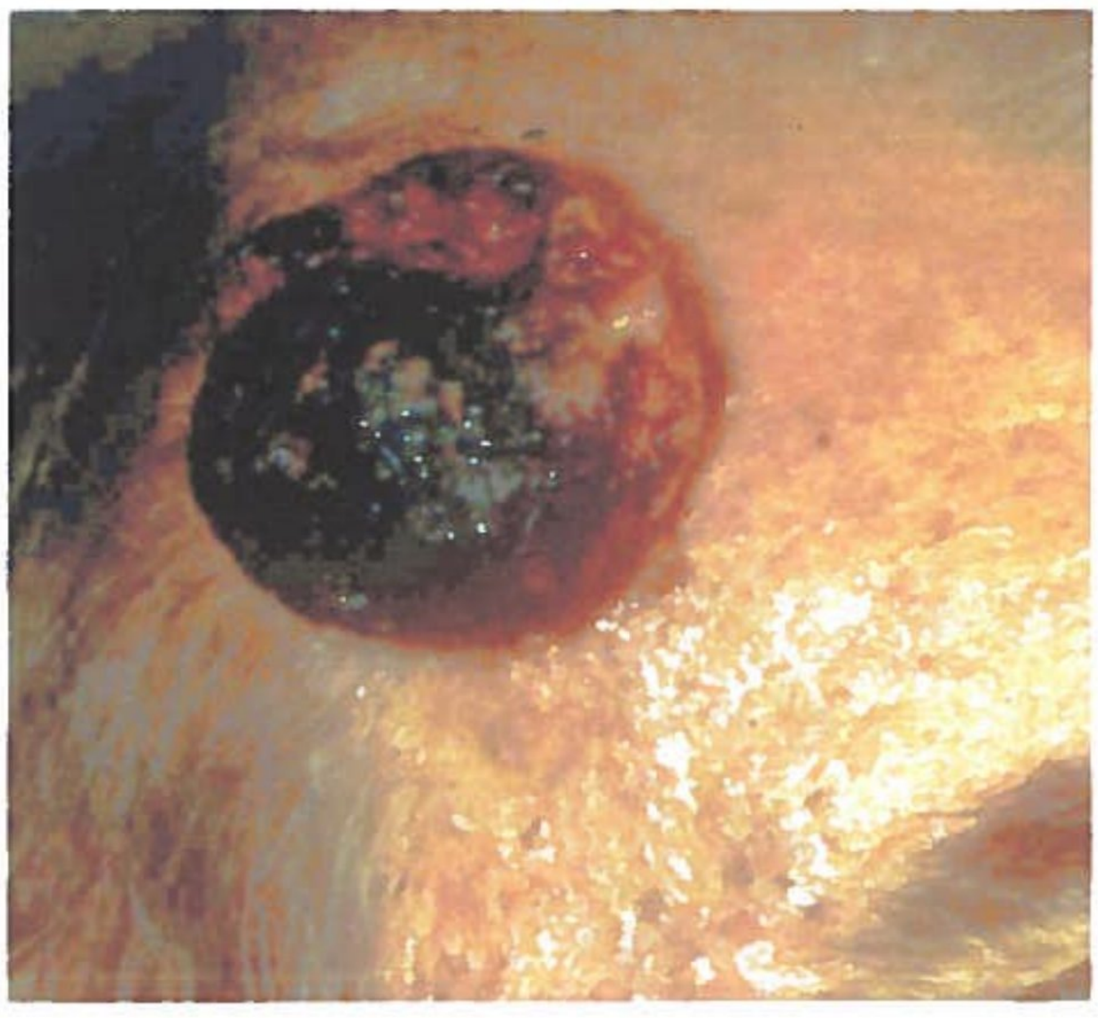


Fig. 1.2 Squamous cell carcinoma in a renal transplant recipient

1.2.1 Time to presentation

The latent period between transplantation and presentation with skin cancer varies from a few months to over 20 years (Table 1). With a few exceptions it appears that the mean latent period is shorter in areas with high levels of sun exposure.

Renal transplant patients develop skin cancers approximately 20-30 years earlier than their non-immunosuppressed counterparts. A study by *Cohen, et al* (Cohen *et al.*, 1987) reported a mean age at presentation with squamous cell carcinoma of 48 years for immunosuppressed patients and 67 years for non-immunosuppressed patients. In the non-immunosuppressed population in the United Kingdom, the mean age at presentation is 70 years for basal cell carcinoma and 73 years for squamous cell carcinoma (Ko *et al.*, 1994).

1.2.2 Relative risks

Calculating the relative risk (i.e. how many times the incidence of skin cancer is increased in the study population in comparison to the general population) was possible only in a few centres that provided control population data. The relative risk of developing skin cancer after transplantation ranged from 3.5 in Sweden

(Blohme & Larko, 1990) to 20 in Australia (Hardie *et al.*, 1980). The increased risk of skin cancer in transplant recipients is almost entirely due to the increased occurrence of squamous cell carcinoma. The increase in the risk of squamous cell carcinoma ranged from 18.4 fold in Canada (Gupta *et al.*, 1986) to 253 fold in the Netherlands (Hartevelt *et al.*, 1990).

The risk of skin cancer is higher in males than in females (Bouwes Bavinck *et al.*, 1996; Gaya *et al.*, 1995) and this is considered to be due to higher levels of sun exposure in males.

The relative risk of basal cell carcinoma was not increased in transplant patients (Gupta *et al.*, 1986; Hardie, 1995; Kinlen *et al.*, 1979) except for a Dutch study that found a relative risk of 10 (Hartevelt *et al.*, 1990). The incidence of malignant melanoma is also increased in transplant patients though at lower levels than squamous cell carcinoma (4.4 fold in Australia (Sheil & (Ed), 1991) and 8.3 fold in the UK (Kinlen *et al.*, 1979)).

1.2.3 Anatomical distribution

The majority of skin cancers occur on sun exposed areas, pointing to the effect of ultraviolet exposure in the pathogenesis (Hartevelt *et al.*, 1990; Liddington *et al.*, 1989). However an increased frequency of skin cancers was noticed in "low risk" areas in renal transplant patients (Liddington *et al.*, 1989). Squamous cell carcinomas are preferentially located on the face and dorsum of hands whereas basal cell carcinomas develop frequently on the face and trunk but were never diagnosed on the back of hands (Hartevelt *et al.*, 1990). A rare site for squamous cell carcinoma development is the eye. Two cases have been published, one of an in situ squamous cell carcinoma of the corneo-scleral limbus (Touzeau *et al.*, 1999) and another one of an aggressive conjunctival squamous cell carcinoma that required removal of the eye (Gaya *et al.*, 1995).

1.2.4 Clinical course

Many patients develop multiple skin cancers of the same or different histological types and cases were reported with more than 100 cancers each (Penn, 1994a). Hyperkeratoses develop frequently on sun exposed sites some of which undergo malignant change and in some patients multiple squamous cell carcinomas arise in these hyperkeratotic areas (Marshall, 1974; Walder *et al.*, 1971).

The dorsum of the hands and forearms can sometime take a characteristic appearance described as "transplant hand" (Fig.1.3). This presents as a

> *"dry and somewhat scaly skin with increasing numbers of either verrucae planae or actinic keratoses, or both" (Blohme & Larko, 1990).*

The changes on the dorsum of the hands can be so severe as to require complete resurfacing with skin graft harvested from a non sun-exposed site (Figures 1.19 and 1.20).



Fig. 1.3 Dorsum of hand showing a combination of solar keratoses and plane warts

Of the 3087 transplant patients with cancer reported from around the world to the Cincinnati Tumour Transplant Registry, 179 (5.8%) developed lymph node metastases (Penn, 1994a). Seventy five per cent of these were from squamous cell carcinomas, 17% from melanomas, 7% from Merkel cell tumours and 1% from basal cell carcinomas. Of the squamous cell carcinomas with lymph node metastases, 99 affected the skin, 18 involved the lips and 17 had both skin and lip involvement. Five per cent of the patients died of their skin cancers with 61% of deaths caused by squamous cell carcinomas, 34% by melanomas, 4% by Merkel cell tumours and 1% (one patient) by basal cell carcinoma (Penn, 1994a). In the United Kingdom only one patient has been reported who died as a result of rapidly evolving metastases from a squamous cell carcinoma on the dorsum of the hand (Glover *et al.*, 1994). The source of the cases is not specified in the Cincinnati Tumour Transplant Registry report. On reading publications from individual transplant centres few cases of metastatic spread and death secondary to skin cancer have occurred in most centres except Australia where metastases occurred in 7% of squamous cell carcinoma and 5% of patients with this tumour died as a result of it. Similarly, 24% of the patients with metastatic melanoma died secondary to their skin cancer (Sheil *et al.*, 1993).

1.2.5 Skin cancers in paediatric renal transplant recipients

Skin and lip cancers in paediatric (less than 18 year old) transplant recipients represented only 19% of the post-transplant malignancies (Penn, 1994b) in comparison to approximately 50% in adult renal transplant recipients. Almost 20% of the skin tumours presented in childhood and of these, half were malignant melanomas.

In addition to malignant melanoma, squamous cell carcinoma and basal cell carcinoma have also been reported. Similar to adult recipients, squamous cell carcinoma was more frequent than basal cell carcinoma. In contrast with the adult recipients, squamous cell carcinomas on the lip occurred more often in paediatric recipients and more squamous cell carcinomas developed lymph node metastases (12% of skin cancers versus 6% of skin cancers in adults). There was a 9% death

rate from skin cancer, which is higher than in adults, the majority of which had lymph node metastases (Penn, 1994b).

1.3 CLINICAL AND HISTOLOGICAL FEATURES OF SKIN CANCERS AND PREMALIGNANT LESIONS

1.3.1 Solar keratoses

These lesions present as localised areas of adherent hyperkeratosis on sun-exposed skin (Fig.1.4). The face, ears, scalp, hands and forearms are usual sites. The lesions may appear as early as 2-6 months after transplantation. In non-immunosuppressed patients the malignant potential of solar keratoses is regarded as low, although a slow-growing squamous cell carcinoma may develop after a prolonged latency. In transplant recipients keratoses tend to be multiple, to recur after conservative treatment and may rapidly evolve into squamous cell carcinomas (Walder *et al.*, 1971).

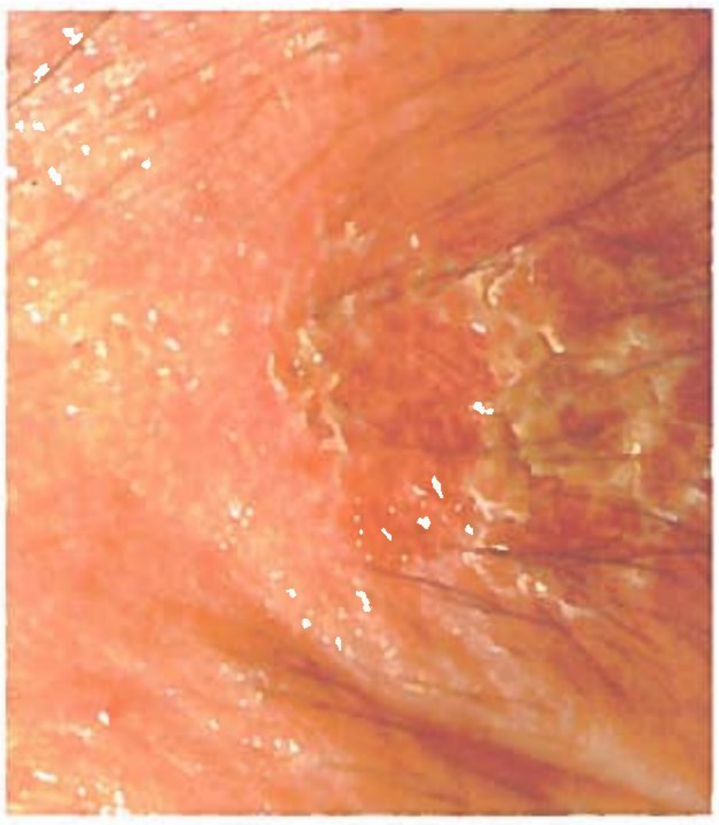


Fig. 1.4 Solar keratosis: yellow scales over an erythematous base.

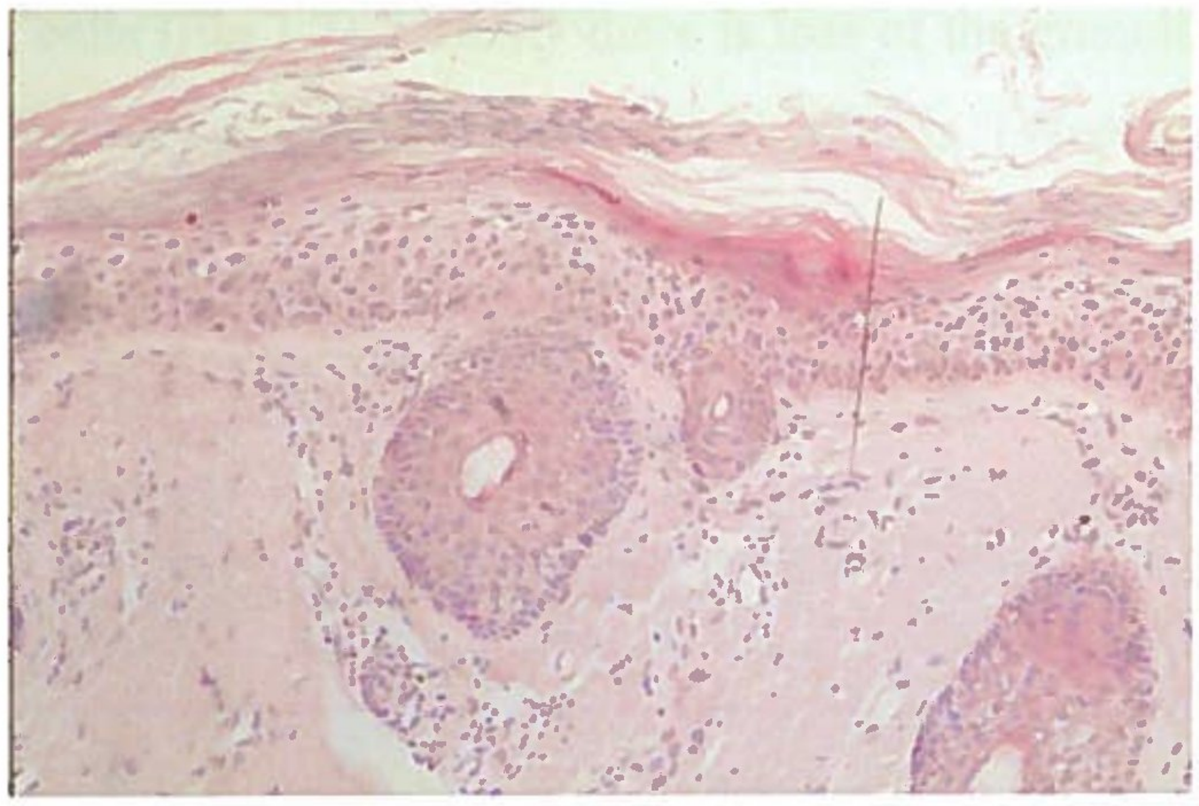


Fig. 1.5 Solar keratosis: alternating hyperkeratosis and parakeratosis associated with epidermal dysplasia (courtesy of Dr. K.Hollowood).

Solar keratoses are characterised by variable loss of the normal orderly stratified epidermal architecture (Fig.1.5) and are composed of keratinocytes showing

varying degrees of atypia. There is also focal parakeratosis, with loss of the underlying granular layer and thickened epidermis with irregular downward buds (Weedon, 1997c).

1.3.2 Bowen's disease

Typically presenting as a persistent scaly erythematous plaque on exposed or covered skin [Fig.1.6]. Bowen's disease represents true carcinoma *is situ* and has undoubted malignant potential. Bowen's disease occurs in transplant recipients, although the frequency is less well documented. McLelland et al reported a prevalence of 5.8% (McLelland *et al.*, 1988). In our experience, the lesions may be clinically atypical and some present as banal keratotic lesions. The differential diagnosis includes solar keratosis, keratoacanthoma, warts or squamous cell carcinoma, all of which may coexist.

On histology atypical-looking cells are present within the whole thickness of the epidermis and sometimes in the pilosebaceous unit. There is also disorderly maturation of the epidermis with mitoses at different levels, multinucleate keratinocytes and dyskeratotic cells (Fig.1.7). Usually there is loss of the granular layer, with overlying parakeratosis and sometimes hyperkeratosis (Weedon, 1997c).



Fig. 1.6 Bowen's disease: persistent scaly erythematous plaque on dorsum of hand.

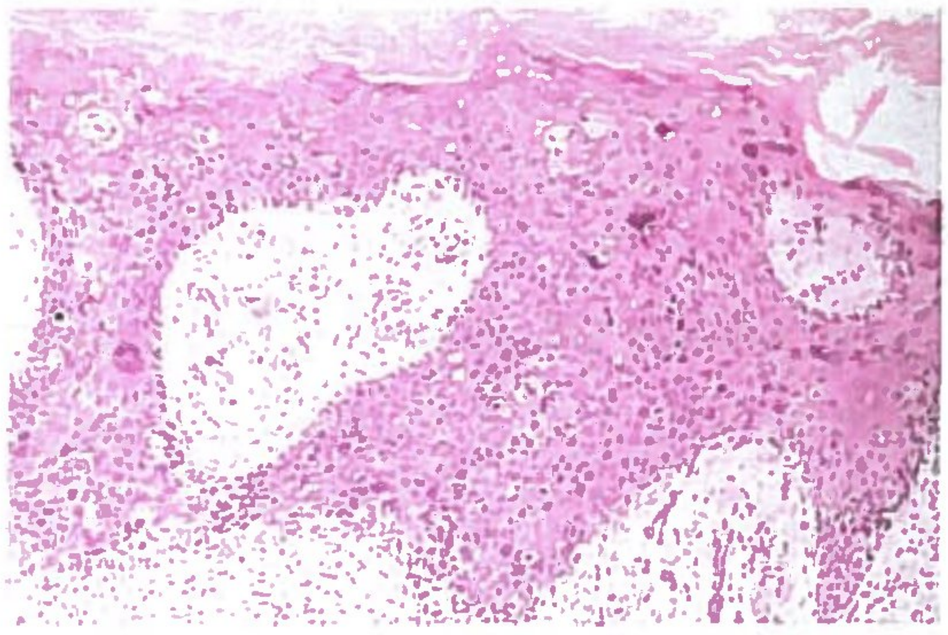


Fig. 1.7 Bowen's disease: Severely dysplastic, irregularly thickened epidermis (courtesy of Dr.K.Hollowood).

1.3.3 Keratoacanthoma

In the non-immunosuppressed patients, keratoacanthoma presents as a firm, rapidly growing, dome-shaped tumour of 1-2.5 cm in diameter with a central keratin-filled crater. They occur mainly on sun-exposed areas but can also develop on any hairy cutaneous site. Keratoacanthomas reach their full size within 6-8 weeks and involute spontaneously generally within less than six months. In some instances they grow for more than two months and take up to a year to involute.

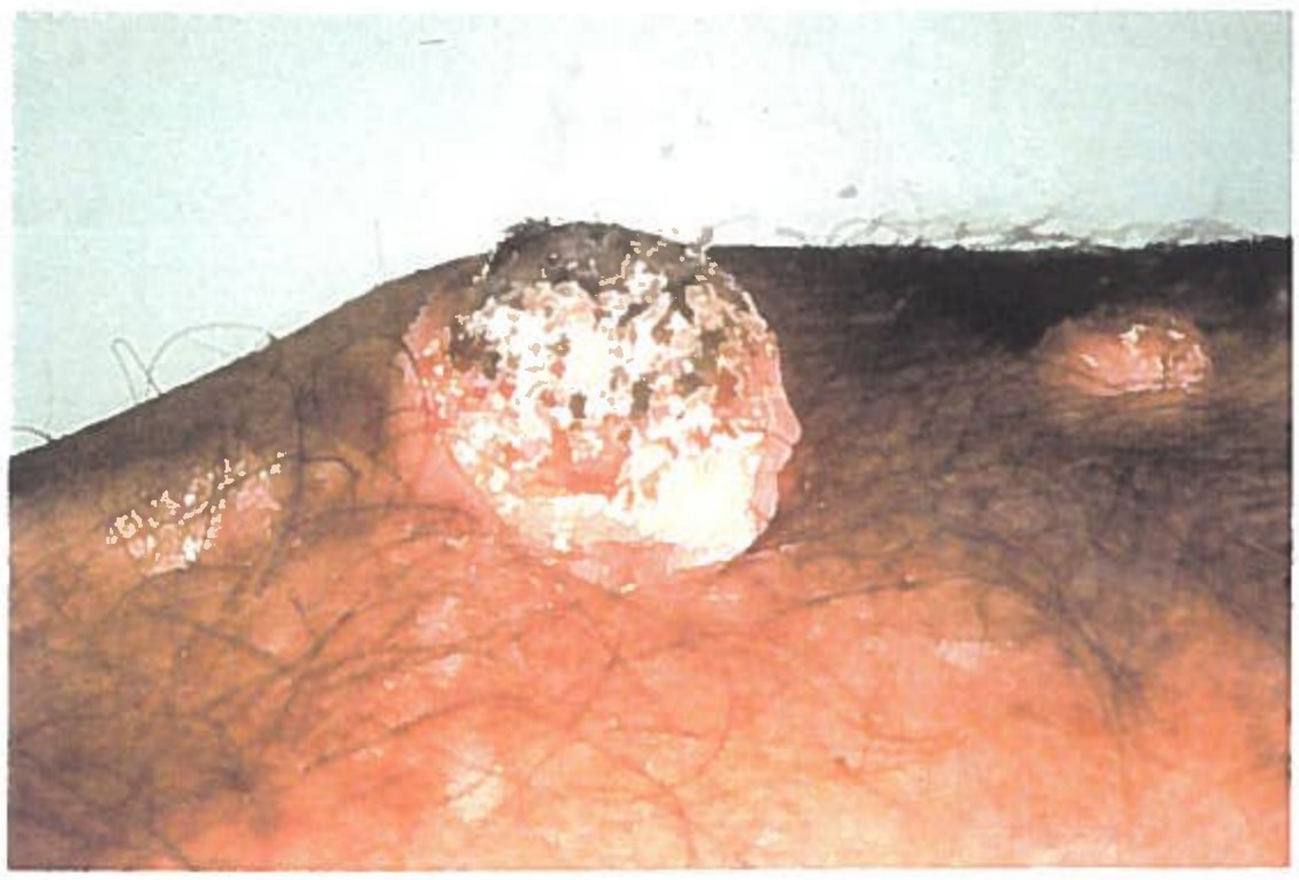


Fig.1.8 Histologically confirmed squamous cell carcinoma presenting as a dome shaped tumour with central keratin plug

Squamous cell carcinomas can masquerade as keratoacanthomas (Fig.1.8) clinically and histologically (Kirkham, 1997) and it is our experience that this happens more often in immunosuppressed, than immunocompetent patients. *Walder, et al* noted in 1971 that

> *"Keratoacanthomas may present the typical appearance but behave in a most atypical manner in these* (transplant) *patients"*

It is difficult to know the true prevalence of keratoacanthomas in renal transplant recipients as some squamous cell carcinomas may have been misdiagnosed as keratoacanthomas.

There is no single consistent histological feature that characterises a keratoacanthoma. They are exoendophytic lesions with an invaginating mass of well-differentiated squamous epithelium at the sides and bottom of the lesion and a central keratin-filled crater. The edges of the lesion overlap the central crater, giving it a symmetrical appearance, a feature that is known as "lipping" [Fig.1.9]. The cells typically have abundant eosinophilic cytoplasm and mitoses are not



Fig. 1.9
Keratoacanthoma: central keratin plug surrounded by collarette (courtesy of Dr. K Hollowood)

common. The degree of cytological atypia is usually mild. Pilar type keratinisation is commoner than epidermoid. Keratoacanthomas do not usually extend below the eccrine glands. In the adjacent dermis there is a mixed infiltrate of inflammatory cells (Weedon, 1997c).

1.3.4 Squamous cell carcinoma

Most squamous cell carcinomas arise in areas of direct sun exposure such as the forehead, bald scalp, face, neck and dorsum of the hands. Commonly the surrounding area will have signs of sun damage: solar elastosis, hyperkeratosis, irregular pigmentation and telangiectasia of the skin or leukokeratosis and fissuring of the lip (MacKie, 1998a). In renal transplant recipients, squamous cell carcinomas usually present as a rapidly growing, raised, keratotic lesion with or without central ulceration, often having an indurated base [Figure 1.10].

Sometimes patients complain of pain or discomfort locally. This is a useful clinical sign pointing to prompt excision especially if occurring recently in a longstanding keratosis that has also increased in size. Squamous cell carcinoma is a true, invasive carcinoma of the surface epidermis, which can spread to the lymph nodes and in some cases cause death.



Fig. 1.10 Squamous cell carcinoma presenting as a sore, raised, rapidly developing keratotic lesion with an indurated base



Fig. 1.11 Squamous cell carcinoma: infiltrating sheets of variably differentiated squamous epithelium with increased mitotic activity. There is proeminent keratinisation. By courtesy of Dr. K Hollowood.

Microscopically the tumour consists of nests of squamous epithelial cells which arise from the epidermis and proliferate downward into the dermis [Fig. 1.11]. The invading tumour nests are composed of atypical cells with eosinophilic cytoplasm

and a large, often vesicular nucleus. There is variable central keratinization and squamous pearl formation. At the periphery of the tumour there is often a mild to moderate inflammatory cell infiltrate (Weedon, 1997c).

1.3.5 Basal cell carcinoma

This is a relatively slowly developing tumour with a tendency to local invasion and tissue destruction although the metastatic potential is extremely low. The prevalence increases with sun exposure. However anatomical distribution does not depend only on sun exposure patterns as this tumour is uncommon on the back of hands and forearms, sites usually exposed to the sun (MacKie, 1998a).

Basal cell carcinomas can present clinically as a papulonodular lesion with pearly translucent edge [Figure 1.12], an erythematous plaque with visible telangiectasia [Figure 1.14], a pale plaque with variable induration. Approximately 2-5% of lesions are pigmented [Figure 1.13]. Unlike squamous cell carcinoma, the clinical appearance of basal cell carcinoma in transplant patients is similar to non-immunosuppressed patients.



Fig. 1.12 Nodulo-ulcerative basal cell carcinoma



Fig. 1.13 Pigmented basal cell carcioma

Basal cell carcinomas are composed of islands or nests of basaloid cells, with characteristic palisading of the cells at the periphery. The tumour cells have a hyperchromatic nucleus with relatively little, poorly defined cytoplasm. There are numerous, sometimes atypical, mitotic figures with high number of apoptotic cells

(Figs 1.15-1.17). The stroma surrounding the tumour islands is newly formed and different from the adjacent dermis. There is a variable inflammatory cell infiltrate composed mainly of T cells, predominantly of the $CD4^+$ subtype. The tumour cells in basal cell carcinoma resemble epidermal basal cells, both in their glycoconjugate pattern, their keratin expression and the presence of *bcl*-2 but they



Fig. 1.14 Basal cell carcinoma on the forehead. Erythema and scaling are present in the superficial variant.

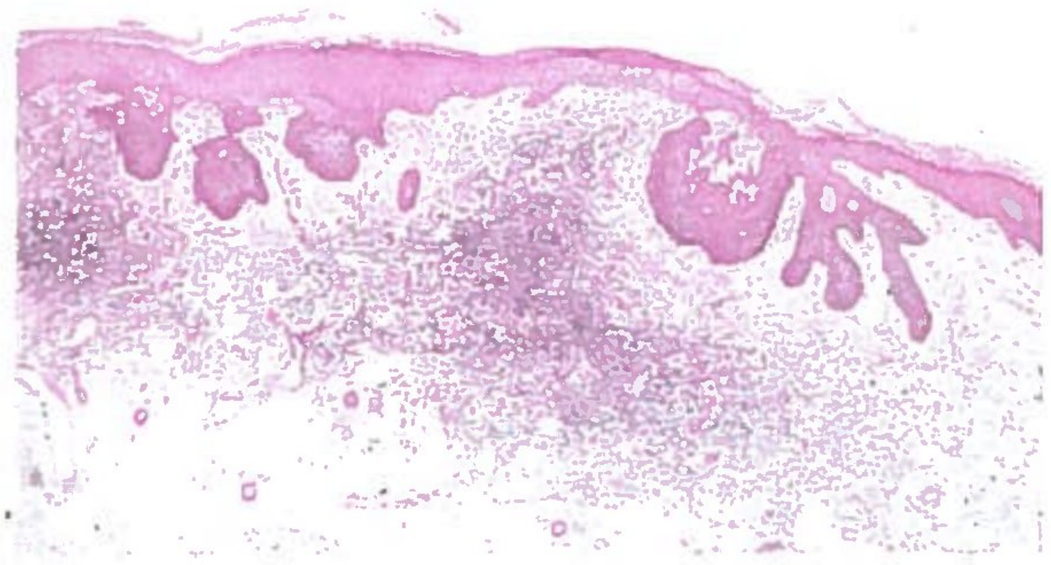


Fig. 1.15 Superficial basal cell carcinoma. Multiple superficial nests with multifocal attachment to the epidermis (courtesy of Dr. K Hollowood)

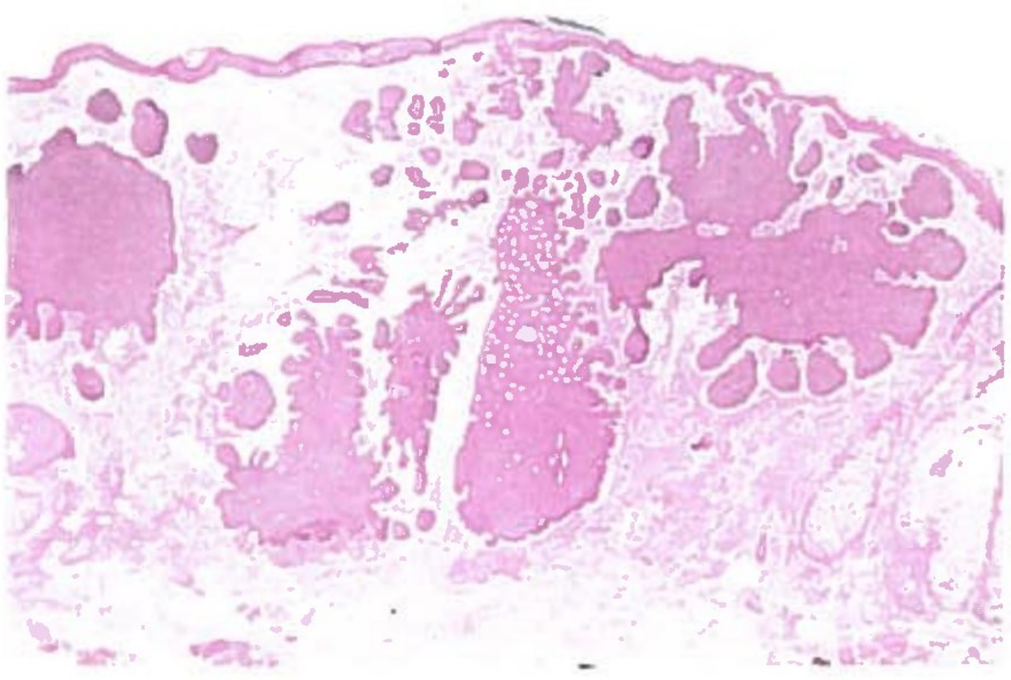


Fig. 1.16 Nodular basal cell carcinoma: large round to oval lobules composed of basaloid cells and showing characteristic artefactual clefting (courtesy of Dr.K Hollowood).

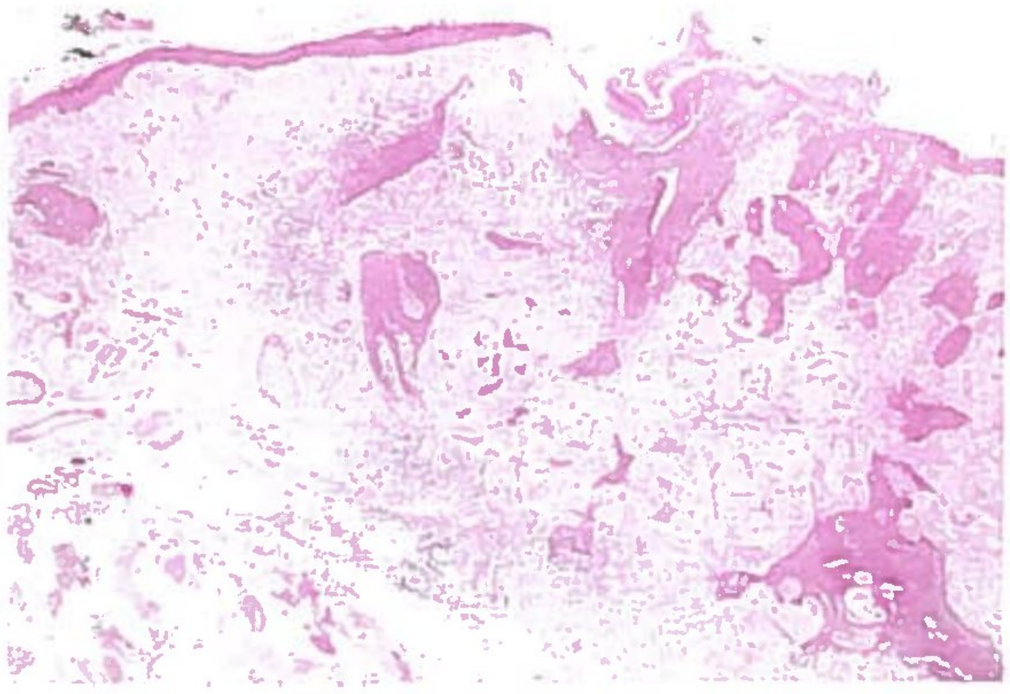


Fig. 1.17 Infiltrating basal cell carcinoma: jagged variably sized nests of basaloid cells set in a fibroblastic stroma (courtesy of Dr. K Hollowood)

also express cytokeratins, which are found only in follicular epithelium (Weedon, 1997c).

1.3.6 Malignant melanoma and dysplastic naevi

Multiple melanocytic naevi erupting 3 months after transplantation have been reported in a patient receiving prednisolone and azathioprine as maintenance immunosuppression and pulsed methylprednisolone for treatment of three acute rejection episodes. The patient had preceding numerous moles, but the newly acquired eruptive naevi were large and somewhat irregular. Some of the lesions showed histological features of dysplastic naevi which in some families are associated with an increased risk of malignant melanoma (Barker and MacDonald, 1988). Another case was reported of an identical twin, who presented 4 years after transplantation with more than 100 small, pigmented naevi on trunk and limbs. None of the naevi looked clinically dysplastic. Prior to transplantation he had only a few naevi similar to his identical twin brother who, subsequently, had no such proliferation of naevi. Maintenance immunosuppression was with azathioprine and prednisolone and methylprednisolone was used for treatment of five acute rejection episodes (McGregor *et al.*, 1991). Many clinically atypical naevi are difficult to distinguish from early malignant melanoma and therefore require excision biopsy and histological examination (MacKie, 1998b).

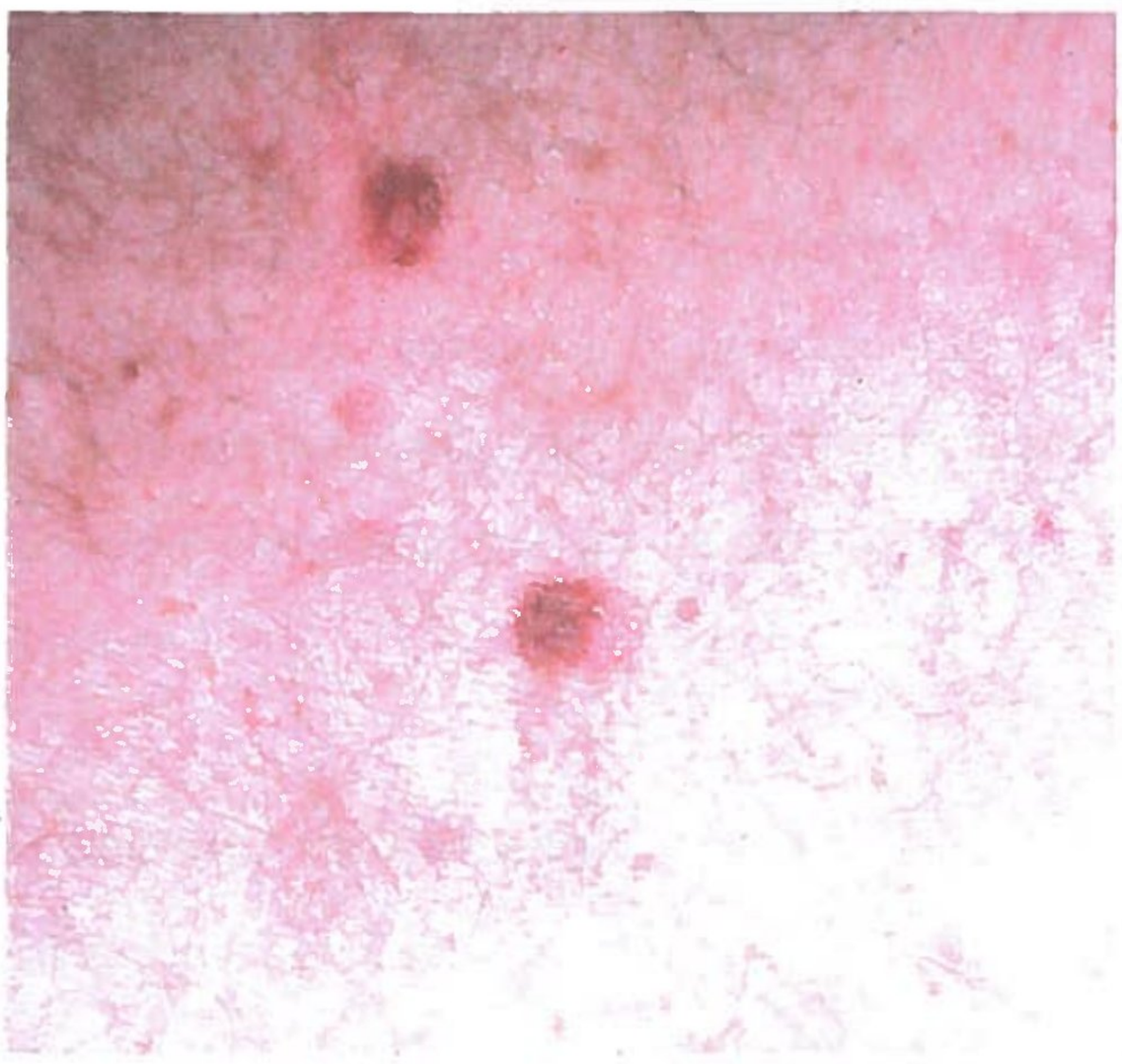


Fig. 1.18 Histologically confirmed dysplastic naevus in a renal transplant recipient who presented with multiple dysplastic naevi after transplantation

Four major clinico-pathologic variants of malignant melanoma are described: superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma and acral lentiginous or palmoplantar melanoma. *Superficial spreading melanoma* presents as an irregularly shaped, brown lesion, usually macular or palpable in later stages, with variation in brown or black pigmentation (MacKie, 1998b). On histology there is proliferation of atypical melanocytes, singly and in nests, at all levels within the epidermis (Weedon, 1997a). *Nodular melanoma* presents as an elevated, dome-shaped, polypoid or pedunculated, reddish brown lesion. Not uncommonly pigmentation may be less prominent and the lesion presents only a peripheral brown ring of melanin surrounding a raised red central area (MacKie, 1998b). *Lentigo maligna melanoma* presents initially as a flat, brown or black, irregularly shaped lesion (Hutchinson's melanotic freckle) which expands slowly (MacKie, 1998b). Invasive malignancy is characterised by thickening of the lesion and development of elevated plaques or discrete nodules. On histology there are atypical melanocytes singly or in nests confined to the basal layer of the epidermis with little pagetoid spread (Weedon, 1997a). *Acral lentiginous melanoma and palmoplantar melanoma* present mainly on the sole of the foot as a large macular, lentiginous pigmented area around an invasive raised tumour (MacKie, 1998b).

1.3.7 Merkel cell tumour

This tumour usually occurs as a solitary reddish blue nodule (MacKie, 1998a) especially on head and neck and extremities (Sibley *et al.*, 1985). Clinically they are often indistinguishable from other skin cancers. The tumour is composed of small to medium cells with scanty cytoplasm and round nuclei, which often have a speckled chromatin pattern (Kirkham, 1997). The three-year survival is 55% (McKee,1996)

1.3.8 Sebaceous carcinoma

Sebaceous carcinoma occurs most frequently on the eyelids but may occur elsewhere on the skin. The tumour is usually solitary, firm, somewhat raised, more or less translucent and covered with normal or slightly verrucose epidermis (MacKie, 1998b). Histologically the tumour consists of lobules or sheets of cells,

showing varying degrees of sebaceous differentiation, separated by fibrovascular stroma (Weedon, 1997b).

1.3.9 Histological features of skin cancers in renal transplant patients versus non-immunosuppressed patients

Clues for differencies in the pathogenesis of skin cancers between immunosuppressed and non-immunsuppressed patients were investigated in tissue sections examined under the light microscope.

Mullen, et al (Mullen *et al.*, 1976) found no histopathological features (level of invasion, histologic grade, tumour pattern, presence of viral inclusion bodies, actinic change in adjacent skin) that distinguished squamous cell carcinomas of the skin and lip in renal transplant patients from similar lesions in non-immunosuppressed patients. The only difference was the significantly reduced inflammatory cell response in the group of transplant patients. These results were confirmed by other authors (Cohen *et al.*, 1987; Glover *et al.*, 1995). Glover et al also looked at Bowen's disease and warts and found an increased number of koilocytes in warts and keratohyaline granules in bowenoid keratoses. The absence of distinctive morphological features in renal transplant patients may indicate that the factors implicated in the development of squamous cell carcinoma in this group of patients are the same as those in the immunocompetent population, the increased rates being attributable to more rapid initiation and progression in the presence of long-term immunosuppression (Glover *et al.*, 1995).

1.4 PRINCIPLES OF MANAGEMENT

Potential renal transplant recipients have to be informed about the risk of skin problems and in particular skin cancers before they decide to opt for organ transplantation. Efforts must be made to reduce sun exposure and patients need to be educated about the dangers of ultraviolet exposure. Additional preventative measures include reduction of immunosuppression to a minimum compatible with

good graft function and regular review of the skin during the lifetime of their transplant, by an experienced clinician.

Non-surgical methods [5-fluorouracil cream, cryotherapy, curretage] can be considered for premalignant lesions and nonspecific keratoses, if a clinical diagnosis can be confidently made. If in doubt, biopsy or surgical excision with



Fig. 1.19 Severe changes on the dorsum of the hand which required excision and reconstruction with full thickness skin graft. Pre-operative view.

histological examination is preferred. The most appropriate treatment for the majority of lesions is surgical excision. This provides histological diagnosis, assessment of aggressiveness and confirmation of the adequacy of excision. Some patients with multiple lesions, especially on the dorsum of the hands may require excision of a larger area and reconstruction of the defect with a skin graft harvested from a non sun-exposed site [Figure 1.6].



Fig.1.20 Postoperative view after excision and reconstruction with full thickness skin graft in another renal transplant recipient

A proportion of renal transplant patients progressively develop widespread and very numerous "warty" skin lesions that include viral warts, solar keratoses,

keratoacanthoma, Bowen's disease and frankly malignant lesions. Such patients may benefit from medical treatment-prophylaxis with topical retinoids (Barranco *et al.*, 1970; Robinson & Kligman, 1975; Rook *et al.*, 1995; Schreiner *et al.*, 1995) or oral retinoids (Bouwes Bavinck *et al.*, 1995; Gibson *et al.*, 1998; Kelly *et al.*, 1991; McKenna & Murphy, 1999; Shuttleworth *et al.*, 1988; Vandeghinste *et al.*, 1992).

Possible mechanisms by which retinoids may reduce the development of skin cancer include inhibition of tumour initiation and promotion, induction of normal cell differentiation and immunomodulation [Holliday, 1992 #264]. Retinoids are known to enhance rejection of skin allografts in mice (Floersheim and Bollag, 1972). However, presumably such potentially adverse effects are overridden by pharmacological immunosuppression because neither etretinate (Shuttleworth *et al.*, 1988) nor isotretinoin (Marcussen & Tyden, 1988; Tam & Cooper, 1987) appears to affect kidney graft function or survival. The antineoplastic effects of retinoids are reversible, and on cessation of treatment new lesions will develop. Due to their side effects retinoids are best reserved for patients with multiple warty dysplstic lesions and malignancies requiring repeated surgical treatment.

Table 1 Studies on the incidence/ prevalence of skin cancer in renal transplant recipients

Authors	Centre	Number of patients	Length of IS[1]	Prevalence/ Incidence	Relative risk[2]	Mean (SD) time to presentation	Tumours recorded; Comments
EUROPE							
Shuttleworth et al., 1987	Cardiff, UK	85	2-20 yr	25%	NA	12.5 yr (5.25 yr)	SK, Bowen's, SCC, BCC
McLelland et al., 1988	London, UK	121	5.9yr (5.2 yr)	9.9%	NA	5.9 yr (5.2 yr)	SK, Bowen's, SCC, BCC, MM
Brown et al., 1988	Belfast, N. Ireland	223	6.6 yr [2-21 yr]	5.8% 0.45/ year	NA	NA	SCC, BCC, KA SCC/BCC = 3.5/1
Liddington et al., 1989	Oxford, UK	598	0-13 yr	5% 3500/100000	NA	7.05 yr [3.6-11 yr]	Bowen's, SCC, BCC, KA, MM
Hartevelt et al., 1990	Leiden, Netherlands	764	8.7 yr [1-21 yr]	6.15% 3.3 BCC/1000py 7.6 SCC/1000py	253 (SCC) 10 (BCC)	9.1 years	SCC, BCC
Blohme et al., 1990	Göteborg, Sweden	98	10-23 yr	37%	3.5	NA	SK, Bowen's, SCC, BCC, Sweat gland Ca, malignant histiocytoma
Vogt et al., 1990	Hannover, Germany	598	3.48 yr [1mo- 5.6 yr]	1 case of SCC lip	NA	NA	SCC
Glover et al., 1994	London, UK	219	4.78 yr [3 mo-21.6 yr]	21.9%	NA	4.78 yr [3 mo-21.6 yr]	Bowen's, SCC, BCC
Thiel et al., 1994	Basel, Switzerland	350	10 yr	17% (CyA group) 15% (Aza group)	NA	NA	SCC, BCC, MM, sarcoma

London et al., 1995	Leeds, UK	918	NA	6% at10 years 30% at 20 years	162 (SCC)[4]	7.75 yr [2mo-19.75yr]	SCC, BCC
Gaya et al., 1995	London, UK	274	2622py	6.9%	19.4	9.5 yr	SCC, BCC, MM
Alamartine et al., 1995	St.Etienne, France	560	NA	3.3%	NA	4.3 yr (2.9 yr)	SCC, BCC
Benalia et al., 1995	Paris, France	915	NA	3.5%	NA	NA	NA
Ferrandiz et al., 1995	Barcelona, Spain	81	1-4 yr	14.8%	NA	1.4 yr [6mo-3yr]	SK, SCC, BCC
AMERICA							
Cohen et al., 1986	Wisconsin, USA	580	12.5 yr	4.5%	NA	6.5 years [9mo-15.8yr]	Bowen's, SCC, BCC, MM, Kaposi's sarcoma
Gupta et al., 1986	Ontario, Canada	523	NA	7.5%	3.2	7.2 yr [2-15.1 yr]	SCC, BCC, MM, Kaposi's sarcoma. SCC/BCC= 2.3/1
Venkateswara et al., 1988	Minnesota, USA	57	1.06 yr (0.18)	10.5%	NA	NA	SCC, BCC
Lugo Janer et al., 1991	Puerto Rico	82	2.9 yr [0.08-13.8]	12%	NA	6.1 yr	SK, SCC, MM (no cases of BCC observed)
Barrett et al., 1993	Ohio, USA	876	3.5 yr	5.36%	NA	7 yr	SCC, BCC, MM, Kaposi's sarcoma, Merkel cell tumour

AUSTRALIA							
Sheil et al., 1993	ANZDATA	6596	NA	20%	NA	NA	SCC, BCC, MM
Hepburn et al., 1994	Christchurch New Zealand	52	9.6 yr (5.9)	19.2%	NA	9.6 yr (5.9 yr)	SCC, BCC,MM
Bouwes Bavinck 1996	Queensland Australia	1098	8.8 yr (5.2)	7% at 1 year 45% at 11 years 70% at 20 years	NA	4.6 yr (4.3 yr)	SCC, BCC, MM SCC/BCC= 1.5/1
OTHERS							
Suzuki et al., 1994	Japan	374	3391py	1.06%	NA	NA	NA

SK – Solar keratosis; SCC – squamous cell carcinoma; BCC – basal cell carcinoma; MM – Malignant melanoma; KA – Keratoacanthoma; yr – year; py – patient years
NA – data not available; [1] mean (SD) or interval [ ] of exposure to immunsuppression; [2] overall relative risk for the skin cancers analysed, unless particular type specified

Chapter 2

Skin Cancers in Renal Transplant Recipients: Pathogenesis

2.1 Introduction

As outlined in the previous chapter, renal transplant recipients are at increased risk of developing skin cancer compared with the non-immunosuppressed population, with squamous cell carcinoma the most commonly diagnosed skin malignancy.

Skin cancers in this group of patients are thought to arise from the combined influences of systemic immunosuppressive drugs, ultraviolet radiation, genetic factors and possibly viral infection, with Human Papilloma Virus (HPV) the principal suspect.

2.2 Ultraviolet exposure

Both in immunocompetent and immunosuppressed patients, the majority of skin cancers develop on sun-exposed areas. Moreover, the incidence of skin cancer is highest in Caucasian patients living in tropical and subtropical climate. Epidemiological studies have shown a relationship between skin cancer and sun exposure. Non-melanoma skin cancers are related to cumulative exposure whereas melanomas (with the exception of lentigo

malignant melanoma) appear to be related to exposure in childhood or intermittent high dose exposure (Young & Walker, 1995).

Most of the ultraviolet rays that reach the skin are absorbed by the epidermis, with transmission of the longer wavelengths only into the dermis. Most studies have been concerned with the ultraviolet (UV) B spectrum, which is considered the most important in mediating immunosuppression but recent data suggests that UVA may also have a significant role, particularly in the development of melanoma (Setlow & Woodhead, 1994). It is believed that UV radiation acts by initiating a cascade of events in the skin starting with absorption by a chromophore or chromophores locally and ending in immunomodulation. Among the first changes, membrane damage, induction of cytoplasmic transcription factors, release of free radicals inducing DNA damage and isomerization of urocanic acid (Fabo & Noonan, 1983; Fabo *et al.*, 1983) appear to play an important part. Both UVB and UVA can induce DNA damage. In a simple model a segment of DNA damage can be repaired successfully if the repair mechanisms are intact. If the repair of DNA damage is unsuccessful, the mutation is preserved and the cell will have one of three possibilities: severe mutations may result in cell death through direct lethality of the mutation or apoptosis; if the mutation has no oncogeneic effect and does not affect phenotype the cell will survive; if the mutation activates oncogenes the cell will become premalignant and later malignant, with the accumulation of further genetic damage.

Ultraviolet radiation can also affect antigen-presenting cells. In human skin it was reported to deplete transiently CD1a+ DR+ Langerhans cells and possibly recruit other CD1a- DR+ leukocytes to the skin while at the same time impairing the ability of Langerhans cells to stimulate proliferative T cell responses in vitro (Baadsgaard *et al.*, 1989). It is therefore possible that antigens encountered through ultraviolet-exposed skin are presented differently, or not at all, from those encountered through normal skin. Changes at the molecular level are induced so that intercellular and intracellular signalling mechanisms are

modified and the local microenvironment becomes more favourable to the development of Th2-like immune responses. These effects can be demonstrated not only at a local level, but at least in rodents, at distant sites also, resulting in antigen-specific immunosuppression. Extrapolating the murine data to humans, it is reasonable to speculate that these defects might further contribute to the breakdown of immunosurveillance already impaired by immunosuppressive drugs and, thus, enhance the development of premalignant and malignant lesions.

2.3 IMMUNOSUPPRESSIVE DRUGS

In order to prevent the rejection of the transplanted allogeneic organ, renal transplant recipients receive long-term treatment with prednisolone, cyclosporine A and/or azathioprine. The mechanisms of therapeutic-induced immunosuppression associated with these drugs have been reviewed by Morris (Morris, 1988) and Walker & d'Apice (Walker & d'Apice, 1988). Cyclosporine inhibits the clonal expansion of T cells by blocking IL-2 synthesis (Fig. 2.1). There is conflicting data regarding the effect of cyclosporine on the expression of IL-2 receptors. Cyclosporine may inhibit the production of IL-1 by macrophages and was shown to inhibit the production of IL-3 and IFNγ. It also inhibits the release of histamine, leukotriene C_4 and prostaglandin D_2. Systemic administration of cyclosporine A in immunosuppressive doses in animal models of chemically induced carcinogenesis caused more malignant squamous cell carcinomas than in animals subjected to carcinogen treatment only (Berger, 1991 #127). In models of UV-induced carcinogenesis systemic administration of cyclosporine or azathioprine increased the incidence and reduced the mean tumour latency period (Berger, 1991). *In vitro* studies have shown that cyclosporine A has the ability to inhibit antigen-processing and accessory functions of epidermal Langerhans cells (Dupuy *et al.*, 1991; Furue & Katz, 1988). *In vivo* studies on Langerhans cells in immunosuppressed patients yielded conflicting results. Some investigators reported no difference in Langerhans cell

distribution in relation to immunosuppression (Kelly *et al.*, 1987; Urabe *et al.*, 1989). Others have found a decrease in the number of Langerhans cells (Bergfelt, 1993; Petzelbauer & Wolff, 1992; Servitje *et al.*, 1991; Sontheimer *et al.*, 1984), detectable as early as 3 days after commencing the treatment (Gabel *et al.*, 1987).

Azathioprine can inhibit both DNA and RNA synthesis and prevent T cell proliferation (Fig. 2.1). *In vitro*, azathioprine also inhibited IL-2 production. Other effects of azathioprine include chromosome breaks (Jensen, 1967) and inhibition of repair of UV-induced damage (Kelly *et al.*, 1986). However the development of tumours in immunosuppressed patients who have not been treated with azathioprine suggests that chemical carcinogenesis is not the only factor involved.

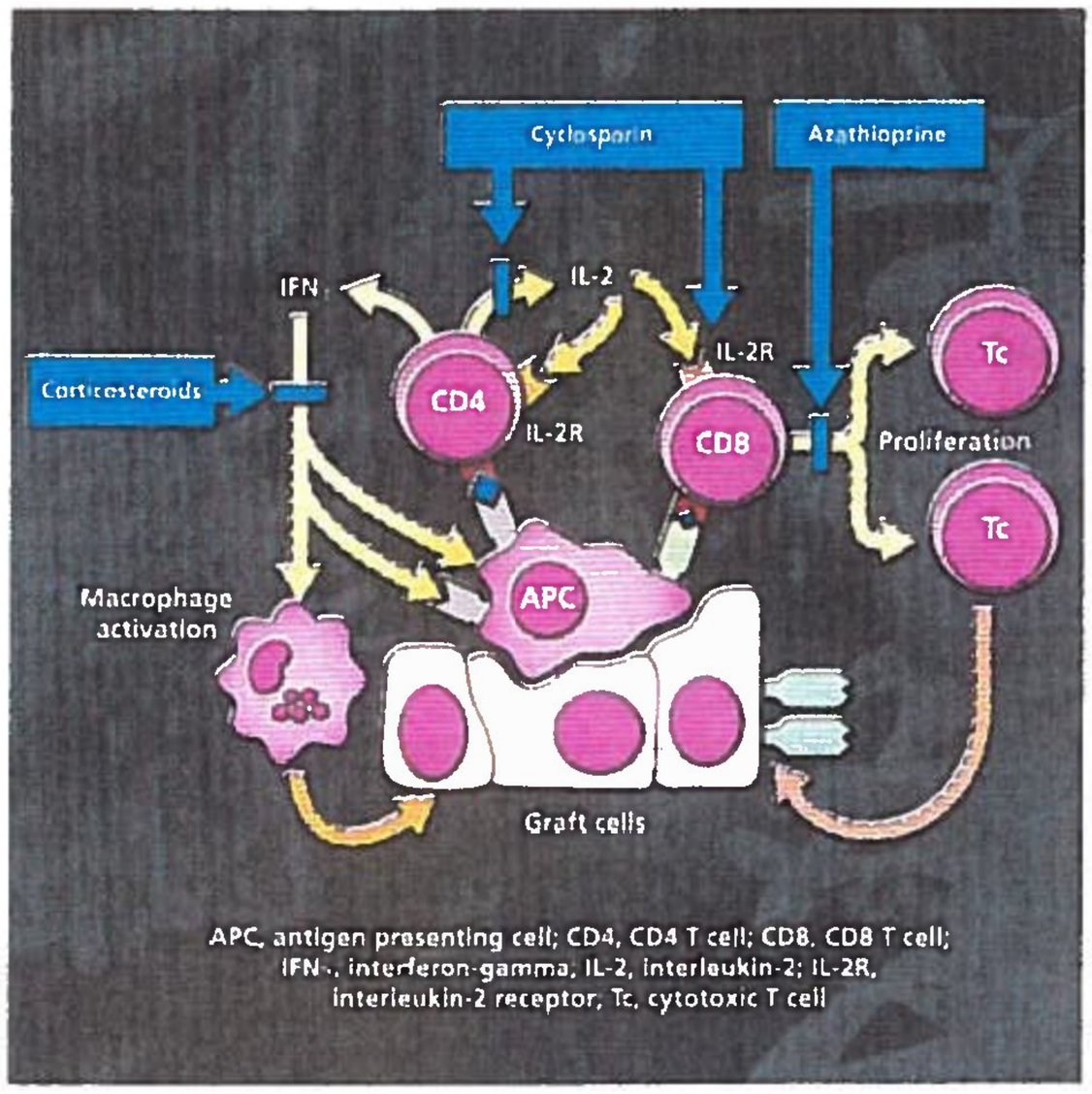


Fig. 2.1 Immunosuppressive drug effects on immune responses (from Novartis – A World of Transplantation leaflet)

Steroids inhibit T lymphocyte proliferation (Dupont *et al.*, 1986) and the inflammatory activity of macrophages and other phagocytic cells. They also deplete epidermal Langerhans cells (Ashworth *et al.*, 1988; Belsito *et al.*, 1982; Berman *et al.*, 1983) and impair their antigen-presenting capacity (Aberer *et al.*, 1984; Ashworth *et al.*, 1988; Belsito *et al.*, 1982). Clinical data suggests that

1984; Ashworth *et al.*, 1988; Belsito *et al.*, 1982). Clinical data suggests that immunosuppression accelerates the UV and HPV induced changes and the effects are significantly reduced when stopping treatment.

There has been much discussion about the risk associated with different immunosuppressive regimens. Disney et al (Disney *et al.*, 1997) found that skin cancer occurred significantly more frequently in patients treated with both cyclosporine and azathioprine than either of this drug alone. In the group of patients treated with cyclosporine only, the incidence was slightly higher for the first six years after transplantation, after which reached similar levels to the group treated with azathioprine alone. In a randomised comparison of two cyclosporine regimens there were significantly more patients who presented with warts, premalignant and malignant skin lesions in the high-dose group than the low-dose group. (Dantal *et al.*, 1998).

2.4 Human papilloma virus

The Human Papilloma Virus has long been recognised as the causative agent of common warts and condyloma acuminata. The Human Papilloma Virus is a small, non-enveloped DNA virus of the papovaviridae family (Shah & Howley, 1996). The virion particle consists of a single molecule of double-stranded circular DNA of approximately 8kb contained within a spherical protein coat, the capsid, composed of 72 capsomeres (Howley, 1996). The viral genome is divided into an *early region* encoding genes required for viral DNA replication and cellular transformation, a *late region* coding for the capsid proteins and a *regulatory region* that contains the origin of replication and many of the control elements for transcription and replication. Of the early region proteins E1 and E2 are involved in viral replication and transcription while E5, E6 and E7 have transforming properties. The viral capsid is made up of two structural proteins, L1 and L2. L1, the major capsid protein contains specific domains involved in the attachment of virus to susceptible cells and probably mediates the immune

responses to viral infection (Shah & Howley, 1996). The function of L2 is not yet clear.

More than 130 HPV types have now been identified, of which 82 have so far been fully characterised (Astori *et al.*, 1998). Viral types are frequently referred to as low- or high-risk depending upon their association with benign or malignant lesions. Specific viral types appear to have a preference for either cutaneous or mucosal surfaces, but a specific clinical lesion may be infected with different HPV types.

The first evidence that HPV infection is associated with squamous cell carcinoma of the skin was found in patients with the rare, genetically determined condition, epidermodysplasia verruciformis. Epidermodysplasia verruciformis is characterised by the development of numerous flat wart-like lesions, which in 30-50% of patients progress to squamous cell carcinomas, 20-30 years later. A specific group of closely related HPV types, the epidermodysplasia verruciformis group, especially HPV 5 and HPV 8 have been isolated from over 90% of squamous cell carcinomas from these patients (Orth, 1987). A role for HPV, analogous to that of epidermodysplasia verruciformis-related skin cancer seems plausible. After transplantation there is an increase in viral skin infections and the tumours with the highest incidence are those thought to arise from oncogenic viruses, Moreover, clinically, squamous dysplasia and squamous cell carcinoma develop in close proximity and are usually preceded by viral wart lesions (Hartevelt *et al.*, 1990). In some patients the skin changes resemble those of patients with epidermodysplasia verruciformis. Histologically, viral warts and keratotic skin lesions often display varying degrees of epidermal dysplasia and some squamous cell carcinomas retain HPV associated features on microscopic examination (Benton *et al.*, 1992).

In 1983, Lutzner, *et al* (Lutzner *et al.*, 1983) reported the identification of HPV type 5 from in-situ and invasive squamous cell carcinomas and viral warts on

sun-exposed skin of a renal transplant recipient (Lutzner *et al.*, 1983). Other groups have later confirmed the finding and reported the presence of other HPV types. With the use of PCR techniques and combinations of primers it is now possible to detect HPV DNA in up to 91% of squamous cell carcinomas (de Villiers *et al.*, 1997). Five viral types (HPV 20, HPV 23, HPV 38, DL40 and DL267) were detected in 73% of the non-melanoma skin cancers tested and were often found in combination with other HPV types within the same lesion (de Villiers *et al.*, 1997). A high prevalence of HPV DNA in renal transplant associated BCC (65%) and SCC (83%) has been reported by other investigators (Surentheran *et al.*, 1998). In addition to epidermodysplasia verruciformis HPV types, which were predominant, mucosal types have also been detected (Surentheran *et al.*, 1998). Recently HPV DNA has also been identified in lesional, perilesional as well as normal skin of non-immunosuppressed patients, although at lower frequencies (de Villiers *et al.*, 1997; Surentheran *et al.*, 1998).

With multiple HPV types present in lesional (not only skin cancer but more recently in psoriatic lesions as well) and clinically normal skin both in immunosuppressed and immunocompetent patients, one might question the functional significance of HPV infection in relation to skin carcinogenesis. It is possible that some skin lesions in renal transplant patients are due to a reactivation of latent HPV infection, which in immunocompetent patients remains quiescent. It is also possible that along with HPV types acquired in childhood, infection with other HPV types occurs after transplantation. Further epidemiological studies are required to establish the prevalence and HPV types present in immunosuppressed versus non-immunosuppressed patients. With regard to mechanisms of viral-induced oncogenesis, the cutaneous types of HPV appear to have different transformation strategies compared with mucosal (genital) HPV types. In particular HPV 8 E6 gene appears not to form complexes with p53, and HPV8 E7 does not bind to the cellular retinoblastoma (RB) protein (Pfister, 1992). At this stage the molecular mechanisms of HPV induced carcinogenesis remain to be established.

2.5 GENETIC FACTORS

Epidemiological data suggests that environmental factors are the most important in the pathogenesis of cancer. However, the response to such factors is determined in part by the genetic make-up of each individual and therefore at one level cancer is a genetic disease. The somatic mutations may occur randomly as the result of imperfect DNA replication or may result from specific gene-environment interactions. A better understanding of the genetic changes involved may enable us to identify individuals who are at risk of developing cancer and perhaps allow us to intervene in the development or progression of the disease.

The somatic mutations may affect two classes of genes: proto-oncogenes and tumour suppressor genes. Proto-oncogenes encode proteins involved in cellular proliferation and/or differentiation including growth factors, growth factor receptors and transcription factors (Weinberg, 1986). Mutation of a single copy of a proto-oncogene can lead to unrestricted cell growth. Tumour suppressor genes control critical checkpoints in cell growth and division and the inactivation of both copies is required for unrestricted cell growth (Knudson, 1993). To these, a new group of genes was added more recently, which are involved in maintaining genomic integrity like the genes encoding for mismatch repair enzymes.

An understanding of the contribution of genes to the pathogenesis of skin cancer came from the study of hereditary syndromes, in particular basal cell naevus syndrome, familial melanoma/ dysplastic naevus syndrome and xeroderma pigmentosum. Both in basal cell naevus syndrome (Gorlin's syndrome or naevoid basal cell carcinoma syndrome) and sporadic basal cell carcinoma mutations can be demonstrated in a gene called *patched* situated on the long arm of chromosome 9: 9q22-23. *Patched* protein serves as a receptor for another protein, *sonic hedgehog*, which controls growth and which forms a complex with another transmembrane protein called *smoothened*. In the absence of *sonic*

hedgehog, patched is bound to and inhibits *smoothened.* When present, *sonic hedgehog* binds to *patched*, resulting in a conformational change in *smoothened* that may result in the activation of transcription factors involved in stem cell proliferation (Halpern & Altman, 1999).

Linkage analyses of familial melanoma/ dysplastic naevus syndrome pointed to the *p16* gene located on chromosome 9. The p16 gene (CDKN2) codes for two distinct proteins, p16 and p19 involved in cell cycle regulation. P16 has been demonstrated to function as an inhibitor of cyclin dependent kinases, CDK4 and CDK6, involved in regulating progression through the G1 phase of the cell cycle (Serrano *et al.*, 1993). Multiple studies have demonstrated germline p16 mutations in familial melanoma kindreds. In addition familial melanoma familes were identified with germline mutations in CDK4, the target of the p16 protein (Halpern & Altman, 1999).

Xeroderma pigmentosum is a rare autosomally recessive disease characterised by early onset of freckling, multiple skin cancers occurring at an early age and other associated abnormalities. Most cases of xeroderma pigmentosum are the result of a heterogeneous group of defects in nucleotide excision repair genes, which are involved in the repair of UV-induced DNA damage (Halpern & Altman, 1999). XRCC3 gene is involved in the double-stranded repair pathway. An allelic variant (exon 7 T/C at position 18067) of this gene has been associated with an increased risk of developing malignant melanoma in a non-immunosuppressed population (Winsey *et al.*, 2000)

Ultraviolet B radiation can damage DNA directly resulting in the formation of covalent bonds between adjacent pyrimidines, generating pyrimidine dimers and 6-4 pyrimidine-pyrimidine lesions (Lear *et al.*, 2000). In addition, both UVA and UVB radiation are able to produce DNA lesions indirectly through oxidative processes involving reactive oxygen species such as superoxide anion, singlet oxygen and hydrogen peroxide (Ananthaswamy & Pierceall, 1990). The negative effect of reactive oxygen species is limited by a complex of enzymes,

of which one group is the glutathione S-transferases (GST). Glutathione S-transferases are grouped in four classes (alpha, mu-GSTM, pi-GSTP and theta-GSTT) with distinct substrate specificities and catalytic efficiencies. Common variants resulting from deletions or single nucleotide polymorphisms have been identified in GSTM1, GSTT1 and GSTP1 genes (Marshall *et al.*, 2000a). In non-immunosuppressed patients the null variant of GSTM1 and the null variant of GSTT1 have been associated with an increased risk of multiple basal cell carcinomas (Heagerty *et al.*, 1996; Heagerty *et al.*, 1994; Lear *et al.*, 1996). GSTP1 Val 105/ Val 105 allele was associated with an increased risk of skin cancers (Ramachandran *et al.*, 2000). In contrast, in immunosuppressed renal transplant recipients, no association was found of the GSTM1 null genotype or GSTT1 null genotype with the risk of either squamous cell carcinoma or basal cell carcinoma while the GSTP1C allele was associated with an increased risk of squamous cell carcinoma (Marshall *et al.*, 2000a).

Factors other than ultraviolet exposure, like smoking, arsenic and soot have been associated with skin cancer. A gene family involved in the metabolism of many chemical carcinogens is the cytochrome P450 (CYP) enzymes. P 450 enzymes are a supergene family with highly polymorphic genes. In addition to gene polymorphism, there can also be wide variations in the basal level of expression between individuals or species and in the inducibility of individual P450 enzymes (Tennant *et al.*, 1999). These enzymes have the ability to insert an atom of molecular oxygen into their substrates, creating an active site for further detoxification by phase 2 enzymes. The human CYP1A gene locus has two members, CYP1A1 and CYP1A2. CYP1A1 is expressed in extrahepatic tissues including skin and is upregulated in response to ultraviolet exposure. A polymorphism in exon 7 was linked with the risk of lung cancer in a Japanese population and with the risk of breast cancer in Caucasians. CYP1A1 m1m1 was associated with increased numbers of basal cell carcinomas (Lear *et al.*, 2000). CYP2D gene locus on chromosome 22 contains three genes. Two genes contain inactivating mutations and the third, CYP2D6 is genetically polymorphic (Lear *et al.*, 2000). In basal cell carcinoma, the CYP2D6 extensive metaboliser

genotype has been associated with an increased number of primary lesions, increased accrual and reduced time to next tumour presentation (Lear *et al.*, 1997).

The p53 tumour suppressor gene on chromosome 17p encodes a 53kDa phosphoprotein with the same name (Lane, 1994). Wild type p53 is involved in arresting DNA damaged cells at the G_1-S boundary (Kastan *et al.*, 1991) and can also cause the removal of such cells by the process of apoptosis (Lowe *et al.*, 1993). It has been suggested that loss of wild type p53 function is an early event in skin cancer (Nagano *et al.*, 1993) and the gene may be a particular target for UV induced damage (Ziegler *et al.*, 1994). There is extensive evidence that p53 mutations/ abnormalities are common events in non-melanoma skin cancer (McNutt *et al.*, 1994). Mutations in p53 were detected in 48% of non-melanoma skin cancers in renal allograft recipients and 63% of sporadic tumours from immune competent patients (McGregor *et al.*, 1997) and increasing p53 reactivity was associated with tumour progression (Stark *et al.*, 1994). The E6 oncoprotein of mucosal types HPV is able to bind to and induce the degradation of p53 through the ubiquitin pathway (Scheffner *et al.*, 1990). A common genomic polymorphism occuring at codon 72 results in the translation of either arginine (Arg) or proline (Pro). The 72Arg form of the protein appeared to be significantly more susceptible to HPV-associated degradation *in vitro* than the proline form (Storey *et al.*, 1998) and it has been suggested that individuals homozygous for arginine 72 are significantly more susceptible to develop HPV-associated cervical squamous cell cancer after transplantation. This result has not been confirmed by other authors (Helland *et al.*, 1998; Hildesheim *et al.*, 1998; Josefsson *et al.*, 1998) or for cutaneous skin cancers after transplantation (Marshall *et al.*, 2000b). It is possible that cutaneous types of HPV do not bind to p53 as it has been shown for HPV 8 E6 gene (Pfister, 1992).

Similar to other medical conditions, certain human leukocyte antigen (HLA) types have been found to be linked with an increased or reduced risk of developing skin cancer (Bouwes Bavinck *et al.*, 1991; Bouwes Bavinck *et al.*,

1997; Czarnecki *et al.*, 1994; Glover *et al.*, 1993; McGregor *et al.*, 1992). The genes of the HLA complex encode specialised peptide-binding glycoproteins present on the surface of the cells, which are involved in antigen presentation to T cells. The genes of the HLA region are highly polymorphic. It is possible that certain alleles are more efficient in presenting the antigen to T cells or the HLA association may be a marker of the effect of other genes in the region on the development of skin cancer.

Chapter 3

Skin Cancer in Renal Transplant Recipients: A Single Centre, 21-Year Retrospective Analysis

3.1 Introduction

Immunosuppressed renal transplant recipients are at increased risk of developing malignancies compared with a non-immunosuppressed control population. In the long-term following renal transplantation the most common malignancies are those of the skin (see Chapter 1). The premalignant and malignant skin conditions occurring most frequently in renal transplant recipients include solar keratosis, keratoacanthoma, basal cell carcinoma, Bowen's disease, squamous cell carcinoma and malignant melanoma.

A number of variables have been investigated in an attempt to identify risk factors for the development of skin cancer after transplantation. Increasing age at transplantation was significantly associated with the risk of skin cancer after transplantation (Alamartine & Bertoux, 1995; Bouwes Bavinck *et al.*, 1996). More males than females develop skin cancer (Bouwes Bavinck *et al.*, 1996; Hartevelt *et al.*, 1990; Liddington *et al.*, 1989) with a relative risk of 0.64 for women compared to men (adjusted for age and number of transplantations) (Bouwes Bavinck *et al.*, 1996). The length of pretransplant dialysis was found to be a risk factor in one study (London *et al.*, 1995). No association was found between the risk of skin cancer and primary renal

disease, donor source (cadaveric or living related), recipient blood group, donor-recipient human leukocyte antigen mismatches, cytotoxic antibodies, additional immunosuppression for rejection episodes (London *et al.*, 1995).

With regard to the risk of skin cancer associated with different immunosuppressive regimens, some authors found an increased risk in patients receiving cyclosporine-based compared with azathioprine-based immunosuppression (Glover *et al.*, 1997; Hiesse *et al.*, 1995; Sheil *et al.*, 1993), while others found no difference (Bouwes Bavinck *et al.*, 1996; Liddington *et al.*, 1989; London *et al.*, 1995; Thiel *et al.*, 1994). In a retrospective study Disney *et al.*, found that skin cancers occurred significantly more frequently in patients treated with both cyclosporine and azathioprine then either of these drugs alone. In a prospective, randomised comparison of two cyclosporine regimens, patients who received the low dose cyclosporine had significantly fewer warts and premalignant/ malignant skin lesions but had more rejection episodes (Dantal *et al.*, 1998).

In non-immunosuppressed patients, smoking was a risk factor for the development of *de novo* squamous cell carcinoma (Aubry & MacGibbon, 1985) or subsequent squamous cell carcinoma in patients with a past history of skin cancer (Karagas *et al.*, 1992).

There have been few single-centre studies on large population samples in Europe that have investigated the incidence, risk factors and clinical features of skin cancer in renal transplant recipients. Moreover, only an earlier report from Oxford (Liddington *et al.*, 1989), included Bowen's disease and keratoacanthoma in the list of recorded skin malignancies. Although multicentre tumour registries include large population samples the accuracy of their epidemiological data especially relating to skin cancer is debatable (London *et al.*, 1995). Our experience in a specialised Transplant Dermatology clinic in Oxford suggested that the incidence/ prevalence of skin cancers after transplantation would be higher then previously reported from

centres in the United Kingdom and Europe. This study aims to be a comprehensive analysis of the epidemiological characteristics of skin cancers occurring in a population transplanted in Oxford over a 21-year period, where nearly all patients have remained under the care of the Oxford Transplant Centre.

3.2 Patients and methods

3.2.1 Patients

From January 1975 until January 1996, 1115 patients received 1360 renal grafts. Of these 1235 grafts were from cadaver donors and 125 from living-related donors. One hundred and thirty-six patients have been excluded from the analysis. These were patients whose clinical notes were not available for review, patients who had been transplanted in Oxford but transferred to another centre before the end of the study period (1 April 1998) and patients who have received their first graft in another transplant centre. This resulted in a total of 979 patients included in the analysis. Total follow up time was 7559 patient-years at risk and individually ranged from 2 to 23 years.

3.2.2 Data collection

Data on skin cancers was collected retrospectively by review of clinical notes, patient charts and histopathology records (see Appendix A.*3 Skin cancer data*). Lesions were recorded that developed both before and after transplantation including the period after graft failure and return to dialysis. In the Oxford Transplant Centre all patients with a functioning graft are seen regularly in the outpatient clinics. Patients with clinically suspicious skin lesions are referred to and then followed up in a dedicated skin clinic within the Transplant Centre. A consultant histopathologist with a special interest in skin pathology undertakes the microscopic examination of all skin lesions excised from transplant patients.

The lesions included in the analysis were histologically confirmed basal cell carcinomas, Bowen's disease, squamous cell carcinoma, keratoacanthoma, malignant melanoma, one case of sebaceous carcinoma and one case of Merkel cell tumour. In the non-immunosuppressed population most keratoacanthomas have a benign clinical course and involute spontaneously. In the immunosuppressed patients, the clinical course of keratoacanthoma resembles more that of squamous cell carcinoma. On histological examination there are features previously associated with both of these tumour types and differential diagnosis between the two conditions can be difficult. Thirty-five tumours were recorded as separate categories; these were 7 basisquamous carcinomas, 9 tumours with full-thickness dysplasia where the possibility of a squamous cell carcinoma could not be ruled out due to the superficial nature of the specimen and 18 tumours with histological features suggestive of both squamous cell carcinoma and keratoacanthoma. In the analysis of the prevalence of different tumour types, these cases together with the Merkel cell tumour and the sebaceous carcinoma were entered in the category named "Others". For each tumour type there was a small number of lesions with no anatomical site specified on the pathology report. These tumours were included in the analysis of the anatomical distribution of skin cancers as a separate category.

In addition to data on skin cancer a number of demographic variables was also collected (see Appendix: A.1 *Demographic variables*). Data on tissue type and other transplant-related variables were kindly provided from the database of the Tissue Transplant Laboratory of the Oxford Transplant Centre (see Appendix: A.2 *Transplant-related variables*). Collected data was entered in an Excel spreadsheet and statistical analysis was performed using SPSS 7.5 for Windows. The immunosuppressive regimen from 1975 to 1978 was azathioprine and high dose steroids. In 1979 cyclosporine was introduced in trials with conventional therapy at the time, followed by triple therapy with cyclosporine, azathioprine and steroids which became the standard regimen in 1985.

3.2.3 Data analysis

The main outcome measure was the development of skin cancer. Differences in the number of tumours, age at transplantation and time to presentation between subgroups were calculated by Pearson chi-square test or Student's t-test. Univariate and multivariate risk factor analysis was carried out using Cox's proportional hazards model with entry and exit criteria set at p=0.05 level. The numbers of person-years at risk was computed from the number of years of immunosuppression for each patient by adding the lifetimes of individual renal grafts. The cumulative incidence of skin cancer was calculated by life table analysis.

Statistical analysis of the distribution of upper limb skin cancer in relation to the side of an arterio-venous fistula was performed using a generalised linear model assuming Poisson errors (Dobson, 1983), with the logarithm of one plus the total number of cancers in each patient as the response variable and an indicator variable for the side of the fistula.

3.3 Results

3.3.1 Descriptive epidemiology

Of the 979 patients included in the analysis, 597 (61%) were males and 382 (39%) were females. Total follow-up time was 7559 patient-years and individually up to 23 years. One hundred and eighty-seven patients developed at least one skin cancer resulting in a period prevalence of 19.1%. Of the 187 patients with skin cancer, 131 (70.1%) were males and 56 (29.9%) were females. Total number of skin cancers was 1065.

3.3.1.1 Skin cancer rates

The rate of any skin cancer was 141/1000 person years at risk. The rates for individual tumour types were:

Squamous cell carcinoma	71.4/ 1000 person years
Bowen's disease	32.5/ 1000 person years
Basal cell carcinoma	22.4/ 1000 person years
Keratoacanthoma	9.26/ 1000 person years
Basisquamous carcinoma	0.93/ 1000 person years
Malignant melanoma	0.53/ 1000 person years
Sebaceous carcinoma	0.13/ 1000 person years
Merkel cell tumour	0.13/ 1000 person years

3.3.1.2 Skin cancer frequencies

The total number of skin cancers was 1065, an average of 5.7 tumours/ patient.

Sixty-eight patients (36.4%) developed only one skin cancer while 119 (63.6%) patients developed multiple skin cancers (Fig. 3.1). In the group of patients with multiple skin cancers 94 (79%) patients have had cancers of different histological types and 25 (21%) patients have had multiple skin cancers of the same histological type. The maximum number of skin cancers excised from one patient was 50. Two patients had 24 different squamous cell carcinomas removed and one patient had 10 basal cell carcinomas removed.

The frequencies of individual tumour types are presented in Table 3.1.

Table 3.1 Frequencies of individual tumour types in renal transplant recipients

Tumour type	**Number of skin cancers/ patient**				
	1	**2-5**	**6-10**	**> 10**	**Total**
	number of patients (%) in each group				
Squamous cell carcinoma	43 (37.1)	42 (36.2)	17 (14.7)	14 (12.1)	116
Bowen's disease	52 (56.5)	25 (27.2)	12 (13.0)	3 (3.3)	92
Basal cell carcinoma	45 (54.9)	33 (40.2)	4 (4.9)	0	82
Keratoacanthoma	15 (44.1)	17 (50.0)	2 (5.9)	0	34

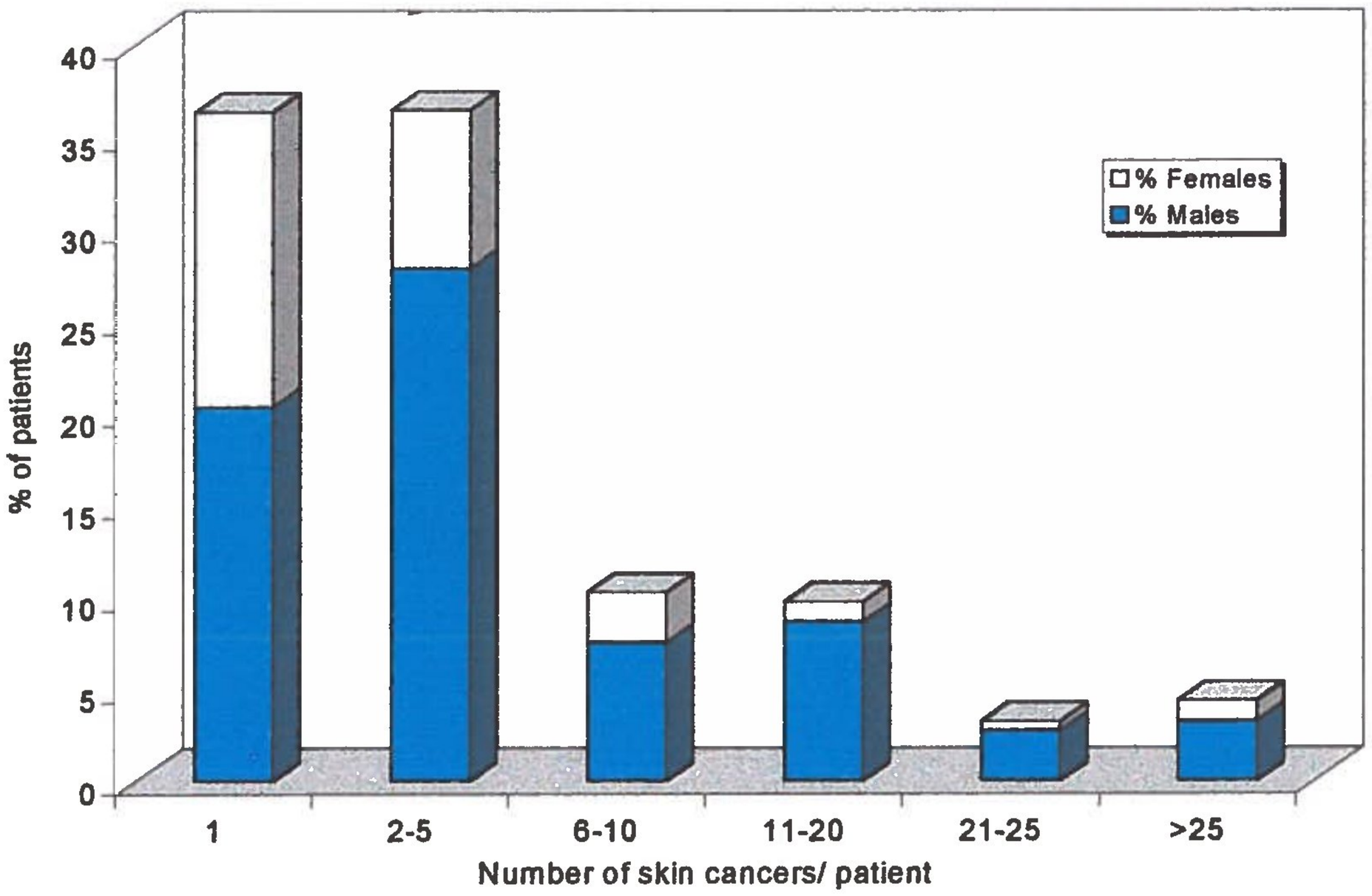


Fig 3.1 Number of skin cancers/ patient in males and females

3.1.1.3 **Histological types** (Figure 3.2)

The numbers on which the percentages are based are given in the Appendix (A.4 *Skin cancers: Histological types*)

Seven patients had basisquamous carcinomas diagnosed, one patient had a Merkel cell tumour and one patient had a sebaceous carcinoma.

There were 119 patients who developed multiple skin cancers, of which 94 patients developed multiple skin cancers of different histological types. The most frequent combination was squamous cell carcinoma and Bowen's disease (Table 3.2).

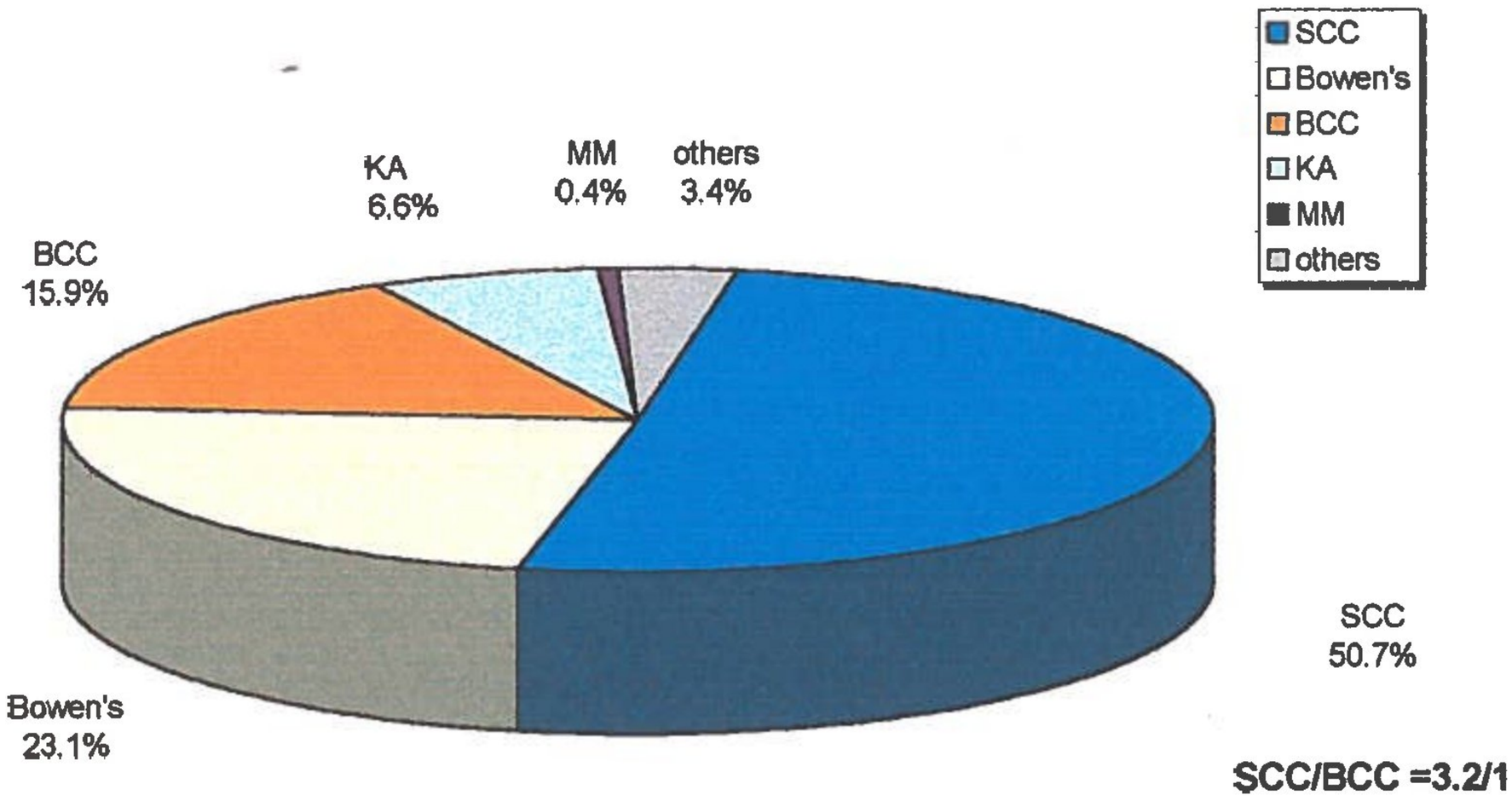


Fig. 3.2 Histological types of skin cancer

Table 3.2 Combinations of skin cancers in patients with multiple lesions of different histological types

Skin cancer types	Number of patients (%)
SCC-Bowen's	**22(23.4)**
SCC-Bowen's-BCC	15(15.9)
SCC-Bowen's-KA-BCC	14(14.9)
Bowen's-BCC	10(10.6)
SCC-Bowen's- KA	7(7.5)
SCC-BCC	7(7.5)
SCC-KA	7(7.5)
SCC-KA-BCC	2(2.1)
SCC-BCC-Basisquamous carcinoma	2(2.1)
Others	8(8.5)
Total	**94**

3.1.1.4 Anatomical distribution

The anatomical distribution of skin cancer in renal transplant recipients is presented in Figure 3.3. The numbers on which the percentages are based and more detailed sub-regional distribution of individual skin cancer types are presented in the Appendix (A.5 *Skin cancers: Anatomic distribution*, A.6 *Skin cancers: Anatomic distribution of individual cancer types*). Sixty-three percent of skin cancers developed on sun-exposed areas (head and neck, dorsum of hands). One sebaceous carcinoma developed on the head and one Merkel cell tumour developed on the neck. Fifty-four percent of the skin cancers occurring on the lower limbs were diagnosed in four renal transplant recipients, all females, who had lower limbs showing signs of lymphatic insufficiency. In addition, in one case, the patient underwent left mastectomy with axillary dissection and radiotherapy for a breast carcinoma and subsequently developed 5 squamous cell carcinomas and one Bowen's disease in the lymphoedematous left upper limb in comparison to only one Bowen's disease in the right upper limb (Bordea *et al.*, 1999). In addition to sun exposure, local factors such as lymphoedema may influence the anatomical distribution of skin cancers.

When anatomical distribution of skin cancers was analysed comparatively in the two genders, males had a higher percentage of skin cancers on the head and neck (43.3% vs 23.3%), hands (23.7% vs 15.8%) and back (6.5% vs 3.9%) compared to females. In turn, females had a higher pproportion of skin cacers on the lower limb compared to males (34.4% vs 3.8%).

3.1.1.5 Time to presentation with the first skin cancer

The mean time interval from transplantation to diagnosis of the first skin cancer was 7.8 years (SD=4.6 years). The earliest tumour presented 3 months after transplantation and the latest one 21 years after transplantation. The mean time to presentation was shorter in patients who developed more than one skin cancer (mean = 7.7, SD= 4.4 years) than in patients who developed

only one skin cancer (mean = 8.2 years, SD = 4.9 years) by the end of the follow-up period. However, the difference did not reach statistical significance (p=0.46)

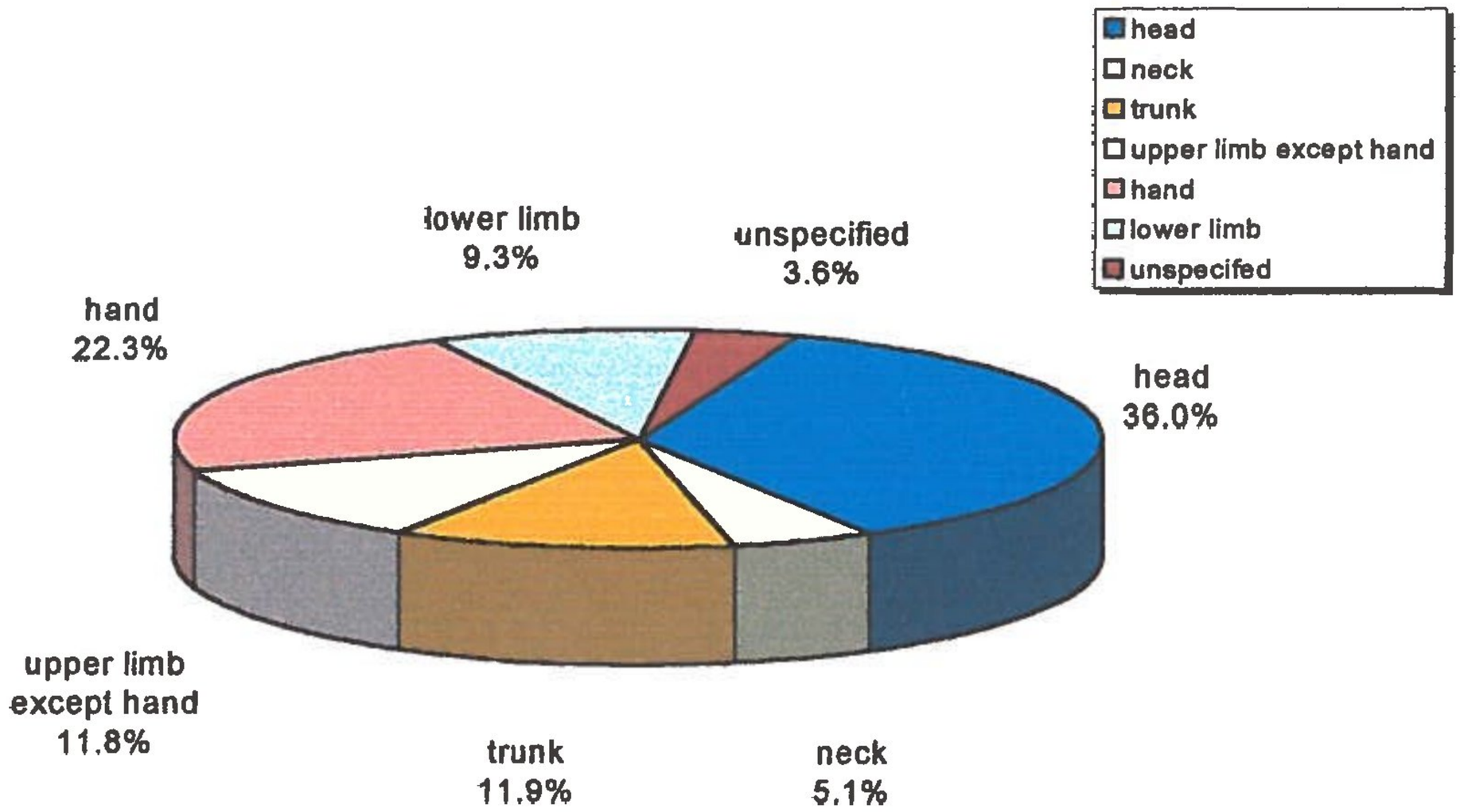


Fig. 3.3 Anatomical distribution of skin cancers

3.1.1.6 Presenting skin cancer

Squamous cell carcinoma was the most commonly presenting skin cancer (36.4% of patients) followed by basal cell carcinoma (21.9%) and Bowen's disease (20.3%) (Appendix A.7 *Histological type first skin cancer presenting*). There were no significant differences in gender, age at transplantation and time to presentation between patients who developed as their first skin cancer a squamous cell carcinoma or a basal cell carcinoma or Bowen's disease (Table 3.3).

Table 3.3 Gender, age at transplantation and time to presentation in relation to type of first skin cancer presenting

Skin cancer type	Gender Number of patients (%)	Age at transplantation Mean (SD)	Time to presentation Mean (SD)
Squamous cell carcinoma	M = 49 (73.1) F = 18 (26.9)	49.6 years (11.6)	8.1 years (4.8)
Bowen's disease	M = 30 (68.2) F = 14 (31.8)	48.8 years (11.2)	8.1 years (4.7)
Basal cell carcinoma	M = 30 (65.2) F = 16 (34.8)	47.4 years (11.2)	6.9 years (4.1)

3.1.1.7 Gender

There were 597 males and 382 females who received a renal graft. Significantly more males (21.9%) than females (14.7) developed skin cancer after transplantation (chi-square=7.67, p=0.007). There were also significantly more males than females who developed multiple skin cancers (chi-square=17.2, p=0.001, Table 3.4).

Table 3.4 Effect of gender on presence and number of skin cancers

	Males Number of patients (%)[1]	Females Number of patients (%)[1]
No skin cancer	467(78.2)	326(85.3)
1 skin cancer	37(6.2)	30(7.9)
2 skin cancers	24(4.0)	6(1.6)
>3 skin cancers	69(11.6)	20(5.2)

[1]Percentage represents proportion of males (females) in each group: 1 skin cancer, 2 skin cancers or more than 3 skin cancers

Females tended to develop the first skin cancer earlier (mean=7.4 years, SD=4.9 years) than males (mean=8.1 years, SD=4.4 years) but the difference did not reach statistical significance (Table 3.3).

3.1.1.8 Age at transplantation

The mean age at transplantation for the entire population was 43years (SD=14 years, range 11-75 years). Patients who developed skin cancer after transplantation were older at the time of transplant (mean=48 years; SD=12 years) than the patients who didn't develop skin cancer (mean=42 years; SD=14 years). Patients who were older at the time of transplantation also developed more skin cancers (chi-square=30.7, $p<0.0001$) and presented significantly earlier ($p<0.001$, see Table 3.4).

3.1.1.9 Skin cancer before transplantation

Five patients had had a skin cancer before transplantation. Three of them had only one skin cancer (squamous cell carcinoma; Bowen's disease; basal cell carcinoma) and the other two had had two skin cancers (two basal cell carcinomas; one basal cell carcinoma and one squamous cell carcinoma) before transplantation. Two of these patients developed further skin cancers after transplantation.

Table 3.5 Effect of age at transplantation on the presence and number of skin cancers and time to presentation

Age at transplant group	Skin cancer group[1] 0	1	2	3+	Time to presentation Mean (SD)
<35 years old	256(89.8)	8(2.8)	4(1.4)	17(6.0)	11.7(5.4) years
35-44 years old	190(80.9)	15(6.4)	7(3.0)	23(9.8)	8.9 (4.0) years
45-54 years old	193(75.1)	28(10.9)	7(2.7)	29(11.0)	7.9 (3.8) years
≥55 years old	153(76.1)	16(8.0)	12(6.0)	20(10.0)	4.3 (2.6) years

[1]Figures represent numbers (%) of patients with no skin cancer, 1 skin cancer, 2 skin cancers and 3+ skin cancers in each age at transplantation group.

3.1.1.10 Lymph node metastases and death secondary to skin cancer

Six patients developed lymph node metastases: two patients with squamous cell carcinoma of the ear, one patient with squamous cell carcinoma of the lip, one patients with a malignant melanoma and each of the patients with sebaceous carcinoma and Merkel cell tumour. The two patients one with sebaceous carcinoma and one with Merkel cell tumour died as a result of their skin cancer.

3.3.2 Distribution of skin cancers on the upper limb in relation to the side of the arterio-venous fistula

Clinical examination of renal transplant patients attending the Renal Transplant Dermatology clinic in Oxford, revealed a small number of patients who had a strikingly increased number of viral warts, keratoses (Figure 3.4) and histologically confirmed malignancies on the side of the arterio-venous fistula. Previously pseudo-Kaposi sarcoma of the hand has been reported to arise in association with an arterio-venous fistula (Goldblum *et al.*, 1985) and an arterio-venous shunt (Landthaler *et al.*, 1989) used for chronic haemodialysis.

We have examined all our renal transplant recipients with upper limb skin cancers and an arterio-venous fistula to determine if there was a true association between the side of the arterio-venous fistula and side of skin cancers on the upper limb (Table 3.6).

Table 3.6 Number of skin cancers in relation to the fistula side

Cancer side	Fistula side[1]		P value
	Left	Right	
Left upper limb	2.15	2.27	0.96
Right upper limb	1.82	2.18	0.31

[1]Figure represents the mean number of cancers



Fig.3.4 Predominant distribution of multiple warts and keratoses on the side of the arterio venous fistula

The difference between the total number of skin cancers that occurred on the fistula-side upper limb and the contralateral limb was not significant.

3.3.3 Risk factors analysis

Thirteen patient-related variables (Appendix A.8 *Cox's univariate analysis of patient-related variables*) and 33 transplant-related variables (Appendix A.9 *Cox's univariate analysis of transplant-related variables*) were tested in Cox's univariate regression analysis to search for association with the risk of developing skin cancer after transplantation. Variables with $p<0.1$ on univariate analysis, variables previously described as risk factors in the literature and other variables of interest were then entered in a multivariate regression analysis.

Multivariate analysis (Table 3.7) showed that recipient age at transplantation is a highly significant risk factor for subsequent skin cancer development (OR=1.07, 95% CI=1.06-1.08, $p<0.0001$). Recipient gender is also a highly

significant factor, female recipients having a lower risk than males recipients (OR=0.51, 95%CI=0.37-0.69, p<0.0001). Transplant recipients who accumulated between 5-10 years of immunosuppression had a risk almost four time higher than patients who received immunosuppression for less than five years (OR=3.9, 95%CI=2.11-7.24). The risk increased to almost ten time in patients with more than 10 years of cumulative immunosuppression (OR=9.81, 95%CI=5.39-17.83). Patients who received a living related kidney graft had a lower risk of skin cancer compared with patients who received a cadaveric graft (OR=0.34, 95% CI=0.14-0.82, p=0.02). Renal function, as reflected by serum creatinine levels (creatinine>150μmol/l) at 1 year was also a risk factor (OR=1.74, 95% CI=1.25-2.43, p=0.001).

The role of different immunosuppressive regimens on the risk of skin cancer development deserves separate discussion. All analyses related to this variable were done for patients with one transplantation only, because the immunosuppressive regimen may be influenced by a history of previous rejected grafts. The proportion of patients with skin cancer in the "cyclosporine era" (16.2%) was significantly lower than in the "azathioprine era" (27%) (chi-square=12.1, p=0.001). Similarly the rate of skin cancer in the "cyclosporine era" (116/1000 person years) was lower than in the "azathioprine era" (192/1000 person years). However when corrected for the time exposure to the risk factors and other risk factors there was no difference between the two regimens (p=0.15).

The ratio of squamous cell carcinomas/ basal cell carcinomas was 4.25/1 in the "azathioprine era" and only 1.94/1 in the "cyclosporine era" (Appendix A.10 *Comparative skin cancer rates and time to presentation in relation to the immunosuppressive regimen*).

The cumulative risk of skin cancer was 9% at 5 years, 27% at10 years, 43% at 15 years and 61% at 22 years after transplantation (Figure 3.5)

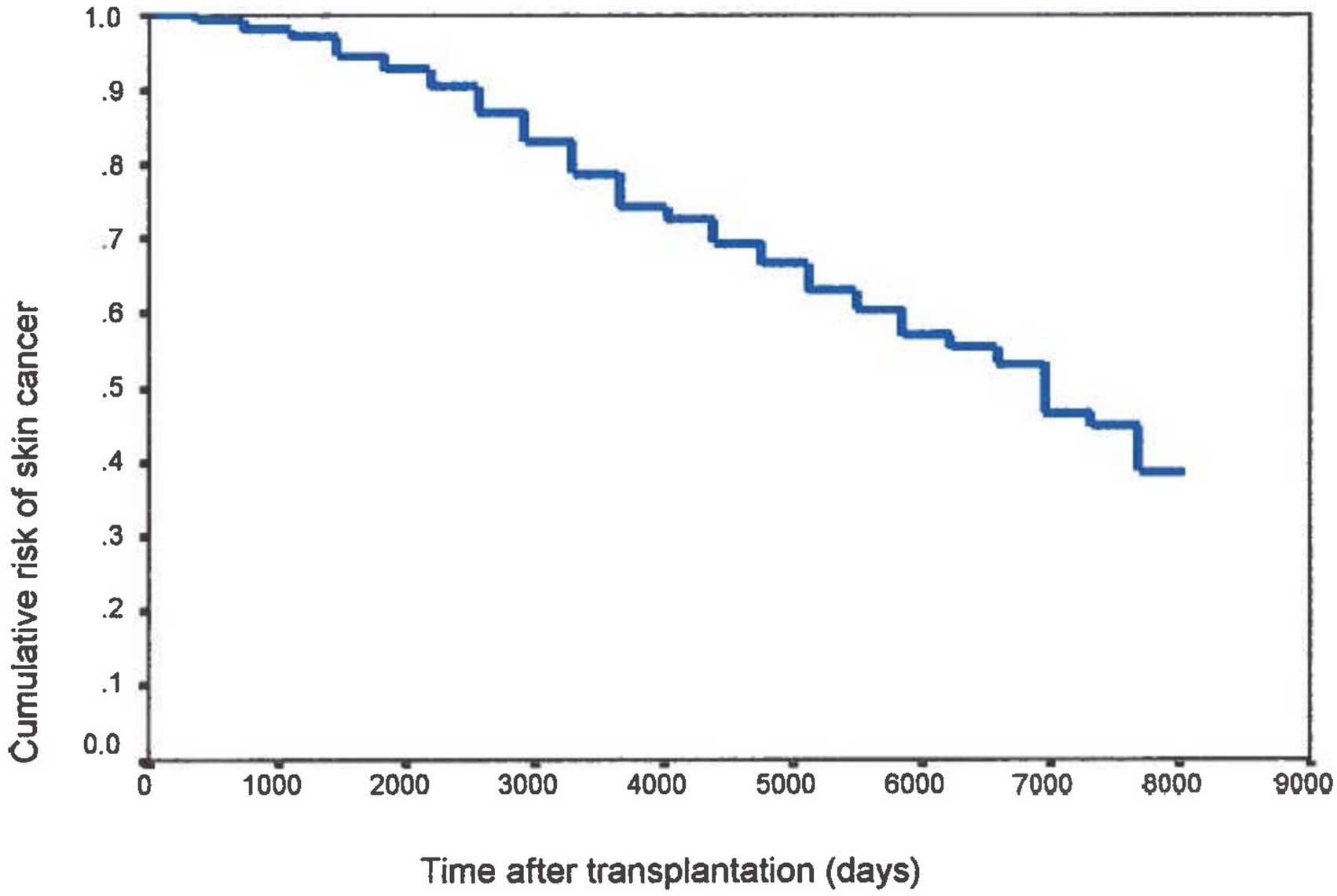


Figure 3.5 Cumulative risk of skin cancer development in the Oxford transplant patients

Table 3.7 Multivariate analysis of factors associated with skin cancer development in Oxford renal transplant recipients

Variable	**Odds ratio**	**95% CI[1]**	**P value**
Age at transplantation	1.07	1.06-1.08	<0.0001
Recipient sex (female)	0.51	0.37-0.69	<0.0001
Total time			
5-10 years	3.90	2.11-7.24	<0.0001
>10 years	9.81	5.39-17.83	<0.0001
Creatinine at 1 year (>150μmol/l)	1.74	1.25-2.43	0.001
Graft relation (living-related)	0.34	0.14-0.82	0.02

[1]CI-Confidence interval

3.4 Discussion

3.4.1 Descriptive epidemiology

The rate of skin cancer after transplantation is higher than previously reported from Europe. The rate of skin cancer in a previous study from Oxford was 35/1000 (Liddington *et al.*, 1989). The rate of skin cancer in the present study, ten years later, was 141/1000. The rate of squamous cell carcinoma was 71.4/1000 and the rate of basal cell carcinoma was 22.4/1000. Hartevelt, *et al* (Hartevelt *et al.*, 1990) previously reported a rate of 7.6/1000 for squamous cell carcinoma and 3.3/1000 for basal cell carcinoma in a Dutch renal transplant population. The rates of other skin cancer types have been calculated and this is the first study to address this aspect.

The period prevalence, which is a less precise statistical measure was 19.1%. This is higher than in studies of similar design (see Table 1.1). The one study that reported a prevalence of 21.6% looked at patients with a currently functioning renal allograft for at least 3 months (Glover *et al.*, 1994). If we select a similar sub-population the prevalence rises to 22.5%.

Two thirds of the patients with skin cancers had multiple lesions and in this group one patient had 50 different tumours removed, with lesions occurring as frequently as once a month. Five percent of the patients with skin cancer had more than 25 tumours. These patients reported that their skin was a cause of continuous concern and the frequent attendance to outpatient and minor operation clinics and the morbidity associated with surgery itself, impacted on their personal and professional life. In the group of patients with multiple skin cancers, 79% of patients had multiple lesions of different histological types. In 23% of cases patients developed a combination of squamous cell carcinomas and Bowen's disease. In a previous study published from Oxford, most frequently patients developed a combination of squamous cell carcinoma, Bowen's disease and keratoacanthoma (Liddington *et al.*, 1989). These results should be regarded as similar. Squamous cell carcinoma can

masquerade as keratoacanthoma both clinically and histologically and it is likely that in the early years some squamous cell carcinomas were diagnosed as keratoacanthomas.

Squamous cell carcinoma was the most common skin cancer type (51%), followed by Bowen's disease (23%) and basal cell carcinoma (16%). Bowen's disease developed in 9% of our transplant population. One previous study reported a prevalence of Bowen's disease in renal transplant recipients of 5.8% (McLelland *et al.*, 1988). Relative risks of Bowen's disease in renal transplant recipients are difficult to calculate in the absence of rates for the non-immunosuppressed population. Four patients developed malignant melanoma resulting in a rate of 53/100000. In the local non-immunosuppressed population (Oxfordshire, Berkshire, Buckinghamshire, Northamptonshire) the age-standardised rate of malignant melanoma was 17.8/100000 resulting in a three fold increased risk in the transplant patients. The relative risk of squamous cell carcinoma was 179 (compared to the four-counties population incidence rate).

The ratio of basal cell carcinoma/ squamous cell carcinoma=1/3.2 is similar to figures published from other centres in Europe (Brown *et al.*, 1988; Hartevelt *et al.*, 1990). In the general population in the United Kingdom, basal cell carcinoma is approximately 4.5 times more frequent than squamous cell carcinoma (Ko *et al.*, 1994). It is interesting to observe that the reversal is less pronounced in Australia (Bouwes Bavinck *et al.*, 1996) and New Zealand (Hepburn *et al.*, 1994). It is possible that higher levels of sun exposure contribute to a higher proportion of basal cell carcinoma whereas in temperate climate squamous epithelial malignancies will develop more often. This could be the result of viral transformation of squamous epithelium facilitated both by iatrogenically suppressed immune responses and sun exposure as reflected in the anatomical distribution of individual skin cancer types.

Similar to non-immunosuppressed patients, most skin cancers (63.4%) occurred on sun exposed areas (head, neck, hand). Twelve percent of skin cancers occurred on the trunk, mostly on the chest and back. Squamous cell carcinoma developed predominantly on the head and hands (70% of tumours). Bowen's disease had a relatively similar distribution on all areas of the body but represented the main skin cancer type of the lower limbs. The most common site for basal cell carcinomas was the face, followed by chest and back. Five percent of basal cell carcinomas developed on the hands and this is a rare site for this type of skin cancer in non-immunosuppressed patients. Keratoacanthomas developed predominantly on the upper limb. The sites of other skin cancer types (malignant melanoma, sebaceous carcinoma, Merkel cell tumour) were similar to those described in the literature.

Fifty-four percent of the skin cancers on the lower limb were diagnosed in four renal transplant patients, all females who had lower limb lymphoedema. In addition one of these patients developed lymphoedema in the left upper limb, following axillary dissection and radiotherapy for a breast carcinoma. Subsequently, she developed 6 skin cancers on the left upper limb and only one skin cancer on the right upper limb. Previously cases of lymphangiosarcoma (Stewart & Treves, 1948), basal cell carcinoma (Benson *et al.*, 1988; Lotem *et al.*, 1994), squamous cell carcinoma (Epstein & Mendelsohn, 1984; Shelley & Wood, 1981), lymphoma (Tatnall & Mann, 1985; Waxman *et al.*, 1984), melanoma (Sarkany, 1972) and Kaposi's sarcoma (Merimsky & Chaitchik, 1992) have been reported to arise in lymphoedematous limbs In these cases the impairment of local immune responses may have contributed to the predominant location of the skin cancers on the lymphoedematous limbs (Bordea *et al.*, 1999).

Squamous cell carcinoma was the most common first skin cancer to present after transplantation. One possible explanation is that squamous cell carcinoma usually arises as a keratotic lesion on sun exposed surfaces, which is more easily noticed by the patients than basal cell carcinoma, which in

24% of cases developed on the chest and back and has a more insidious clinical course. However, the time to presentation for patients who first developed a basal cell carcinoma was shorter (6.9 years) than in patients who first developed a squamous cell carcinoma (8.1 years) or Bowen's disease (8.1 years). Similarly there was no significant difference in the ages at first transplantation or gender distribution between patients presenting with, squamous cell carcinoma, Bowen's disease or basal cell carcinoma (Table 3.3).

The mean age at transplantation in the group of patients with skin cancer was 48 years and the mean age at presentation with skin cancer was 56 years. The resulting 8 years time to diagnosis of the first skin cancer is similar to that reported from the Netherlands – 8 years (Hartevelt *et al.*, 1990), but is longer than in Australia - 4.6 years (Bouwes Bavinck *et al.*, 1996). Higher levels of sun exposure may account for the shorter time to development of skin cancer after transplantation in the Australian population. In the non-immunosuppressed population in the United Kingdom, the mean age at presentation is 70 years for basal cell carcinoma and 73 years for squamous cell carcinoma (Ko *et al.*, 1994). Thus renal allograft recipients present with skin cancer approximately 15-20 years earlier.

The risk of skin cancer in females was 0.51 that of males. There was also a male predominance in the group of patients with multiple skin cancers (Table 3.4). Females tended to present earlier (7.4 years) than males (8.1 years) although the difference was not statistically significant. The higher risk of skin cancer in males is thought to be related to higher levels of sun exposure. When the anatomical distribution was analysed separately in males and females it became apparent that males had a higher proportion of skin cancers on traditionally male-sun exposed sites like head and neck, hands and back possibly related to occupational or recreational sun exposure. Females had a 10-time higher proportion of skin cancers on the lower limbs compared to males.

Patients who are older at their first transplantation have a significantly higher risk of skin cancer afterwards ($p<0.0001$). They developed multiple skin cancers and presented with the first skin cancer significantly earlier than patient who were younger at their first transplantation (Table 3.5).

Of the five patients who had had a skin cancer before transplantation, two developed up to two skin cancers after transplantation and none of them has had lymph node metastases or died secondary to their skin tumour. In the Australian experience patients with a history of pre-transplant skin cancer had an increased incidence of skin cancer after transplantation, but without any increase in mortality (Bouwes Bavinck *et al.*, 1996). While it is difficult to draw conclusions based on our limited experience it seems reasonable to affirm that in a country with low levels of sun exposure, a history of pre-transplant skin cancer need not be considered a contraindication to transplantation.

Lymph node metastases developed in six patients who had skin cancers that are known to have metastatic risk (squamous cell carcinoma of the ear or lip, malignant melanoma, Merkel cell carcinoma, sebaceous carcinoma) even in non-immunosuppressed patients. Two (0.2%) patients, one with sebaceous carcinoma and one with Merkel cell tumour died secondary to their skin cancer.

3.4.2 Distribution of skin cancers on the upper limbs in relation to the side of the arterio-venous fistula

There was a higher mean number of skin cancers on the left upper limb (2.15) than on the right upper limb (1.82) in patients who had an arterio-venous fistula on the left forearm, but this did not reach statistical significance. In patients with a right forearm arterio-venous fistula the mean number of skin cancers on the right upper limb (2.18) and left upper limb (2.27) were similar. We have been unable to confirm the initial clinical observation of a possible

relationship between the side of the arterio-venous fistula and the side of skin cancers on the upper limbs.

3.4.3 Risk factor analysis (Appendix A.8, A.9 and A.10)

Age at first transplantation was a highly significant risk factor (OR=1.07, 95% CI=1.06-1.08, p<0.0001) for the development of skin cancer after transplantation. The skin cancer risk increase (adjusted for other risk factors) was 7% for each additional year of age. Patients who were between 35-44 year old had a 2.5 time higher risk than patients who were less than 35 year old at first transplantation. The risk increased linearly with age and patients who were more than 55 year old had a 9.5 time higher risk than patients who were under 35 year old at first transplantation.

Female recipients had 51% of the risk (adjusted for the other risk factors) of male recipients (OR=0.51, 95% CI=0.37-0.69, p<0.0001).

Graft survival and total time exposure to immunosuppression were highly significant risk factors (p<0.0001). The risk was almost 10 times higher in patients who had been exposed to immunosuppression for more than 10 years compared to patients exposed for less than 5 years.

Patients with serum creatinine levels above 150 μmol/L, at 1 year after transplantation, had a higher risk of developing skin cancer. One possible explanation is that these are patients with less than optimal graft function possibly due to rejection and would have had additional immunosuppression. Patients who received antilymphocyte immunoglobulin (p=0.03) or OKT 3 (p=0.05) had a higher risk of skin cancer, but the associations became non-significant (p=0.9 and p=0.5 respectively) after correction for other risk factors. There was no increase in risk associated with administration of Methylprednisolone (p=0.2). It is possible that quantifying the total dose of additional immunosuppression rather than the presence or absence would be a better way of testing this hypothesis.

Patients who received their renal transplant from a living-related donor had 34% of the risk (adjusted for other risk factors) of patients who received their graft from a cadaver (p=0.02). In most cases of living related donation matching for HLA was a selection factor and these patients required lower cumulative doses of immunosuppressive drugs to prevent rejection.

Overall there were no associations between the number of HLA mismatches and the risk of skin cancer. This result does not support the hypothesis that chronic antigenic stimulation from the graft facilitates oncogenesis [Penn, 1977 #288] unless antigens other than the ones tested may important.

There was no association with the cause of renal failure, type or length of dialysis, recipient blood group or smoking status. Smoking was previously associated with the risk of *de novo* squamous cell carcinoma (Aubry & MacGibbon, 1985) or subsequent squamous cell carcinoma in patients with a past history of skin cancer (Karagas *et al.*, 1992). It is possible that in renal transplant recipients the effect of smoking is overridden by other, more important risk factors.

With regard to the risk of skin cancer associated with either azathioprine or cyclosporine we found no difference between the two regimens, when adjusting for the other risk factors. All analyses relevant to this question were done for patients with one transplantation only. The rate of skin cancer was higher in the azathioprine "era" (192/1000 person-year) than in the cyclosporine "era" (112/1000 person-year). This increase is mainly due to a higher rate of squamous cell carcinoma (103/1000 person-year versus 50/1000 person-year). The rates of basal cell carcinoma (24/1000 person-years versus 26/1000 person-year) and Bowen's disease (39/1000 person-year versus 34/1000 person-year) were similar in the two periods. In absolute numbers there were more basal cell carcinoma and Bowen's disease excised in the cyclosporine "era" than azathioprine "era". This may be due to the longer time exposure to immunosuppression or possibly, better follow up and

earlier detection of more insidious lesions. The "better follow-up" explanation is further supported by a shorter time to presentation in the cyclosporine "era" (5.2 years) compared to the azathioprine "era" (10 years)

The ratio of squamous cell carcinoma to basal cell carcinoma was higher in the azathioprine "era (4.25/1) than in the cyclosporine "era" (1.94/1). As this is a ratio of absolute numbers of tumours, the difference is due to a higher number of squamous cell carcinomas in the azathioprine "era". It is possible that in addition to general immune unresponsiveness azathioprine acts through other specific but different mechanisms that would promote preferentially the development of squamous cell carcinoma.

The cumulative risk of skin cancer at 22 years was 61%. This is higher than previously reported from Europe: 40% Hartevelt, *et al* (Hartevelt *et al.*, 1990) and 30-40% London, *et al* (London *et al.*, 1995). Part of the difference may be due to the fact that Hartevelt, *et al* only recorded basal cell carcinoma and squamous cell carcinoma and the number of skin cancers in the study of London, *et al* was small. It is also due to more rigorous follow up and data collection in our study.

Chapter 4

The Role of Immunological Factors in the Aetiology of Squamous Cell Carcinoma in Renal Transplant Recipients

4.1 Background

Experimental data from animal studies as well as clinical and laboratory data from patient studies suggest that the immune system is important in the response to tumours. The incidence of cancer is higher in patients receiving therapeutic immunosuppression than in control populations. Anecdotal reports of "spontaneous" regression of tumours would indicate that in certain conditions the body is able to activate mechanisms that restrict tumour development. Regressing skin tumours have an associated inflammatory cell infiltrate and in the case of melanoma, infusion of ex-vivo expanded tumour-infiltrating lymphocytes induced regression (Rosenberg *et al.*, 1990).

4.1.1 The skin immune system

The skin is the largest organ of the human body, and its principal function is that of a barrier. In addition, the skin has a complicated defence function that allows it to deal with the variety of exogenous substances to which it is exposed [reviewed in (Bos *et al.*, 1997)]. In 1986, Bos proposed the term *Skin*

Immune System to describe both cellular and humoral constituents that are involved in the immune responses to antigens (Bos & Kapsenberg, 1986).

The immune response-associated cells are keratinocytes, Langerhans cells, T lymphocytes, vascular and lymphatic endothelial cells, tissue macrophages, monocytes, tissue dendritic cells, mast cells and fibroblasts. The non immune-associated cells are Merkel cell, melanocytes, fibrocytes, pericytes, eccrine glandular cells and apocrine glandular cells, sebocytes, Schwann cells, smooth muscle cells. In addition to the cellular constituents of the skin immune system, a wide variety of inflammatory and immune mediators are present within the normal integument.

In an adaptive immune response naïve T cells proliferate and differentiate into armed effector T cells that are able to act rapidly upon encounter with MHC-peptide complexes on antigen presenting cells. Antigens i.e. viral proteins processed in the cytosol are presented to CD8+ cytotoxic T cells in the groove of a MHC class I molecule. Antigens processed in intracellular vesicles, from ingested bacteria and toxins are bound to MHC class II molecules and presented to CD4+ T cells. Upon encounter with the MHC class II–antigen complex, CD4+ T cells proliferate and differentiate into one of the two types of effector cell: inflammatory T cells (T_H1) or helper T cells (T_H2). T_H1 cells activate infected macrophages to destroy intracellular pathogens. T_H2 cells activate B cells to produce antibodies (Janeway & Travers, 1996a).

Cytotoxic T cells are important in controlling virus infections including those caused by oncogenic viruses (Gooding & O'Connell, 1983; Kast *et al.*, 1989). Tumour-specific cytotoxic T cells have also been demonstrated in tumours not caused by viruses (Crowley *et al.*, 1991; Wolfel *et al.*, 1989). Although CD8+ cytotoxic T cells are thought to be primarily responsible, CD4+ T cells can also have cytolytic activity. In some viral induced tumours CD4 T cells participate in antigen recognition by CD8 cells. In these cases both the naïve CD8 cell and the CD4 T cell must recognise antigen on the surface of the same antigen-presenting cell. The role of CD4 T cells is to compensate co-

stimulation by the antigen-presenting cell that is inadequate for direct activation of the CD8 cells (Janeway & Travers, 1996b). CD4 T cells can also enhance tumour destruction by activating macrophages. In an experimental murine leukaemia virus induced tumour model in mice, tumour destruction was mediated by macrophages that had become tumoricidal after activation by cytokines secreted by tumour-specific CD4+ T cells (Greenberg *et al.*, 1988). However CD8+ T cells recognising tumour-derived peptides in the groove of MHC class I molecules are the most powerful mediators of tumour destruction (Melief & Kast, 1991).

MHC class I molecules are found on all nucleated cells. MHC class II molecules are normally expressed on B-lymphocytes, dendritic cells and macrophages. The keratinocytes can be induced to express MHC class II molecules (Lampert, 1984) suggesting that they may have some activity as an accessory cell in T cell stimulation, but do not express B7-1 or B7-2, ligands for CD28. The inability of keratinocytes to deliver co-stimulatory signals to T cells may be responsible for their apparent lack of antigen presenting activity. However, the keratinocytes have the ability to modulate the immune responses through their secretion of cytokines such as IL1, TNFα, IL12 and possibly IL10 (Luger, 1981 #296; Kock, 1990 #297; Aragane, 1994 #298; Enk, 1992 #299).

The major antigen presenting cells in the skin are the Langerhans cells. They are dendritic-shaped cells which are located in the basal and suprabasal parts of the epidermis. Langerhans cells have the capacity to uptake antigen (Shelley & Juhlin, 1976; Shelley & Juhlin, 1977), process it and present the antigen to T lymphocytes (Braathen & Thorsby, 1980; Stingl *et al.*, 1981; Stingl *et al.*, 1978). In response to locally released inflammatory mediators such as TNF or chemokines, LCs increase their surface expression of MHC class II (Larsen *et al.*, 1990) and co-stimulatory molecules such as B7-1 (CD80) and B7-2 (CD86) (Larsen *et al.*, 1994). They also increase in size and change shape. The same stimulus promotes migration of LCs in afferent

lymphatics as veiled cells which then home to the draining lymph nodes where, as potent immunostimulatory dendritic cells, they activate naïve (CD45RA+) T cells. This leads to the development and clonal expansion of antigen-specific memory (CD45RO+) T cells, which circulate via the blood. Langerhans cells are responsible for the presentation of antigen during the induction phase of contact hypersensitivity reactions. Studies by Steiner et al. (Steiner *et al.*, 1985) and Grabbe et al. (Grabbe *et al.*, 1991) suggest that LCs may also be able to activate immunological defence mechanisms against neoantigens and tumour antigens.

4.1.2 Immune responses to allergens and irritants

Allergic contact dermatitis is a common dermatological condition, which is considered to be a form of delayed hypersensitivity. When the relevant allergen is applied to the skin of sensitive patients an eczematous reaction resembling the disease is elicited over a period of one to three days (Holgate & Church, 1993). Contact sensitisers are relatively small chemical molecules (haptens) that have to bind to a protein carrier to be immunogenic, forming a hapten-carrier complex. They usually bind to host proteins and modify them chemically. The modified self-proteins are then cleaved into modified self-peptides, which can bind to MHC class II and are transported to lymph nodes and presented to CD4+ T cells. Re-exposure of the skin to the same contact allergen induces upregulation of adhesion molecules expression on keratinocytes in the epidermis and endothelial cells in the dermis, which promotes the influx of memory T cells to the site of skin contact (Bos *et al.*, 1997; Janeway & Travers, 1996a). The activated cells are generally type 1 CD4+ cells producing IFNγ and TNFα. The released inflammatory cytokines increase vascular permeability allowing the extravasation of fluid and proteins and recruitment of inflammatory cells to the site of contact (Kapsenberg, 1992 #300). IFNγ also induces HLA DR expression on a variety of cells including macrophages, keratinocytes and fibroblasts rendering these cells suitable to present antigen to T cells (Basham, 1985 #301; Kameyama, 1987 #302). It is

uncertain whether LCs are essential for the elicitation phase of contact dermatitis.

Induction of contact hypersensitivity responses by skin allergens appears to be dependent on the density of LCs. Induction of contact hypersensitivity was reduced in skin areas with low densities of LCs induced by UVB irradiation, stripping of epidermal tissue or chemicals (Rheins & Nordlund, 1986; Saunder *et al.*, 1981; Stingl *et al.*, 1981).

A clinical presentation similar to the allergic dermatitis may be elicited by the application of an irritant. Irritants (e.g detergents, oils) are substances which, when applied locally, are capable of inducing a nonspecific local inflammatory reaction. It usually occurs on the hands and is not a hypersensitivity response, as no sensitisation is required. Patch testing of the irritant only produces a non-eczematous irritant reaction in all patients (Holgate & Church, 1993). Irritants capable of inducing acute irritant contact dermatitis show enormous variation with respect to their physico-chemical properties and consequently act on the skin by a variety of different mechanisms. In the majority of cases there is some degree of intercellular oedema and spongiosis in the epidermis, though less pronounced than in allergic contact dermatitis. Most irritants also induce intracellular vacuolation and nuclear pyknosis within epidermal keratinocytes. There may also be acantholysis, hydropic swelling, parakeratosis and dyskeratosis. Exposure to irritants also appears to alter keratinocyte surface antigen expression, in response to irritant-mediated cytokine release and as a consequence of direct irritant induction. There is contradictory data with regard to upregulation of MHC class II, ICAM-1 and CD 36 by irritants. The role of the LC is as yet unclear. There have been contradictory reports on the effect of irritants on LCs densities, partly explained by the use of different chemicals and other variations in experimental design. Whether the changes to LCs have a functional significance or represent cellular toxicity has yet to be determined. In the dermis there is disruption and/ or degeneration of collagen, oedema and

an inflammatory infiltrate of mononuclear cells similar to that seen in allergic contact dermatitis. CD4+ T cells are in majority, accompanied by CD8+ T cells, macrophages, CD1+ T cells and neutrophils. B cells, natural killer cells and follicular dendritic cells are only rarely observed (Willis, 1996).

4.1.3 Immune responses to skin cancers

In Bowen's disease, an intraepithelial skin cancer, an inflammatory infiltrate is present in the upper dermis, consisting principally of T cells and HLA-DR+ large non-dendritic cells of the macrophage/ monocyte lineage, with CD4+ T cells predominating over CD8+ T cells. In specimens with moderate or heavy inflammatory cell infiltrate, both CD4+ and CD8+ T cells invaded the tumour and were observed in the vicinity of tumour cells (Habets *et al.*, 1989).

In basal cell carcinomas the majority of cells that infiltrate the tumour islands are T lymphocytes (Habets *et al.*, 1988). Basal cell carcinomas have been reported to regress spontaneously (Curson & Weedon, 1979; Franchimont *et al.*, 1982). Immunohistochemical studies found a significantly increased number of CD3+ and CD4+ T cells infiltrating basal cell carcinomas showing histological signs of regression, compared to non-regressing basal cell carcinomas (Hunt *et al.*, 1994).

Keratoacanthoma, which shares clinical and histological similarities with squamous cell carcinoma, presents as a rapidly growing lesion which reaches its full size within 6-8 weeks and usually involutes spontaneously within less then six months (Kirkham, 1997). The quality of the inflammatory infiltrate in a keratoacanthoma varies with the stages of evolution. In the early stages the infiltrate is sparse with few lymphocytes at the interface between the advancing margin and the reticular dermis. In established lesions the infiltrate is dense and in foci migrates into the columns of cells to produce lytic clefts with necrotic keratinocytes and loose infiltrates of lymphocytes and histiocytes (Reed, 1990). Comparative immunohistochemical studies found that keratoacanthomas had a greater number of apoptotic cells, a greater

degree of tumour degeneration and a heavier peritumoral mononuclear cell infiltrate than squamous cell carcinomas. They also had a higher density of CD3+ cells, predominantly of the CD4+ phenotype with a greater number of these cells staining positive for the IL2 receptor (Patel *et al.*, 1994).

Tefani et al., found a significant increase in the number of CD3+ T cells, with a predominance of CD4+ cells in spontaneously regressing malignant melanomas compared to rapidly growing and metastatic melanoma where CD8+ cells were predominant (Tefany *et al.*, 1991).

Delayed type hypersensitivity responses to dinitrochlorobenzene (DNCB) or recall antigens, as indicators of the cell mediated immune response, have been investigated in non-immunosuppressed patients with basal cell carcinomas (Czarnecki *et al.*, 1996), melanoma (Morris *et al.*, 1979), head and neck cancers (Mandel & Kiehn, 1974), breast cancer (Cunningham *et al.*, 1976). Reduced responses to antigens correlated with poor prognosis or increased risk of multiple malignancies. In a recent study de Berker *et al.*, found that non-immunosuppressed patients with squamous cell carcinomas had reduced responses to DNCB compared to age and sex matched normal controls (de Berker *et al.*, 1995).

In immunosuppressed organ transplant recipients testing for delayed type hypersensitivity responses to either DNCB or recall antigens has been used to assess cell-mediated immune responses during chronic renal failure (Chaterjee, 1985) or to predict graft survival (Schaafsma *et al.*, 1989; Westphal *et al.*, 1986). Responses to recall antigens were studied in renal transplant patients with and without cancer. A significant proportion of transplant patients had no clinical response compared to age and sex matched normal controls. There was also a trend to an increase in the number of non-responders in the cancer group compared with the non-cancer group of transplant patients (Lodge, 1995). The reduced immune responsiveness in transplant patients is iatrogenically induced through the administration of

immunosuppressive drugs such as cyclosporine, azathioprine and corticosteroids in order to prevent graft rejection (reviewed in Chapter 2).

4.2 STUDY DESIGN: PATIENTS AND METHODS

The study aimed to compare in vivo delayed-type hypersensitivity responses to new antigens (DNCB), recall antigens and contact allergens and inflammatory responses to irritants in the following groups of patients:

Group 1 Renal transplant recipients with a history of three or more squamous cell carcinomas.

Group 2 Renal transplant recipients without a history of skin cancer, matched with patients in group 1 for gender, age at transplantation, type of graft (cadaveric/ living related), duration and type of immunosuppression.

Group 3 Normal controls age and gender-matched with patients in groups 1 and 2. Age matching between groups 1,2 and 3 was done within 10 years of age.

Group 4 Non-immunosuppressed patients with one or more squamous cell carcinomas.

Group 5 Normal controls age (within 10 years) and gender-matched with patients in group 4.

The Central Oxford Research Ethics Committee granted approval for the study with regard to both testing and methods of recruitment of cases and control patients (COREC 96.184).

4.2.1. Identification of cases and controls

Renal transplant recipients under the care of the Oxford Transplant Centre, who had three or more squamous cell carcinomas, were identified from the skin cancer database. Letters of invitation (Appendix A.11 *Letter of invitation*

(1)) to take part in the study accompanied by information leaflets detailing the tests used (Appendix A.12 *Information leaflet on skin tests* and A.13 *Information leaflet on skin samples*) and a schedule of the visits were sent to potential cases identified. A few weeks later the patients were approached to explore their interest in the study. Not all the patients who fulfilled the criteria to enter the study agreed to participate, mostly due to time commitments elsewhere and this limited the number of cases in the study. Transplant patients without a history of skin cancer were identified from the same skin cancer database. In addition patients' notes and drug charts were studied, to match for the type and duration of immunosuppression. Before entering the study all patients were offered the opportunity to ask more questions and were asked to complete a consent form (Appendix A.14 *Form of consent*)

Non-immunosuppressed patients with a history of squamous cell carcinoma were identified from the records of the Histopathology Departments in Oxford and Stoke Mandeville. Before approaching the patients a letter was sent to their general practitioner to check if there was any history of malignancy, immunosuppressive drug treatment, radiotherapy, or any other medical conditions or social circumstances that would have prevented them from taking part in the study. The initial aim was to recruit non-immunosuppressed patients with three or more squamous cell carcinomas. The patients who fulfilled this criterion had ages between 80-90 years and most were unfit to take part. It also posed us with the problem of comparing groups of patients with greatly different mean ages and interpreting the results. Ageing itself is associated with reduced responses to recall antigens and contact allergens, particularly in subjects over the age of 75 [reviewed in (Sunderkotter *et al.*, 1997; Thivolet & Nicolas, 1990)]. We have therefore chosen to select patients who had at least one skin squamous cell carcinoma. Patients were sent an invitation letter (Appendix A.15 *Letter of invitation (2)*), information leaflets on the study (Appendix A.12 *Information leaflet on skin tests* and A.13 *Information leaflet on skin samples*), a reply slip (Appendix A.16 *Patient reply slip*) and a stamped self-addressed envelope. Patients who replied were

then contacted via their preferred route (telephone/ post). This gave them a chance to ask further questions on the study and allowed us to explore briefly the past medical history. Patients who decided to take part in the study were then invited for the first visit when detailed clinical and sun exposure histories were taken. Control groups were selected among people who volunteered to help in the study. Posters were put up in all the major hospitals in Oxford together with brief information sheets (Appendix A.17 *Patient information leaflet*), reply slips and reply envelopes. Patients who declared an interest in the study were sent further detailed information (as above) and another reply slip. Only when this second reply slip was received were patients contacted. The past medical history and in particular skin history were briefly explored and their permission was asked to write to their general practitioner to inform them about the study and check the medical history.

Patients were excluded if there was a history of: UV therapy; skin cancer; eczema; lymphoma or leukaemia; long term use of topical or inhaled steroids; treatment with azathioprine, cyclosporine or other immunosuppressive or cytotoxic drugs; radiotherapy. One patient who had used 1% Hydrocortisone cream intermittently and for short periods was accepted on condition that he would not use the cream from at least a week before DNCB sensitisation until after the 96-hour readings. In most clinical situations it can be assumed that all the drug has been effectively eliminated after three to four half-lives [Winter, 1994 #318]. The percutaneous absorption of hydrocortisone varies from 1% to 40% depending on the anatomical site and the biological half-life in term of anti-inflammatory action is 8-12 hours (1999).

4.2.2 Methods

4.2.2.1 Clinical studies

The ability to mount a delayed type hypersensitivity response was tested for a new antigen (DNCB), recall antigens and contact allergens. In addition we assessed inflammatory responses to the irritants dithranol and sodium lauryl

sulphate. The choice of doses used for testing was based on previously published data and the results of a small pilot study.

A. 2,4 dinitrochlorobenzene (DNCB)

DNCB is a highly potent contact sensitiser that has been used for studies of cell-mediated immune responses. Studies by Friedman *et al*. (Friedman *et al*., 1989), and Rees *et al.* (Rees *et al*., 1990), have shown that 30μg of DNCB applied on a 1cm patch test, for 48 hours sensitise 85% of the subjects. In an attempt to sensitise 100% of the subject we used for sensitisation 40μg of DNCB. Ten microlitres of a 0.4% (w/v) solution of DNCB (Crawford Pharmaceuticals Ltd, Milton Keynes, UK) in acetone (Heylings *et al*., 1996) were pipetted on a filter paper disc in an 11mm Finn Chamber (Epitest Ltd, Helsinki, Finland) (Rees *et al*., 1990). This was applied on the inner arm. For transplant patients who had had an arterio-venous fistula for haemodialysis the non-fistula arm was chosen for sensitisation. In the case of one transplant patient who had had mastectomy and axillary dissection for a breast carcinoma the contralateral inner arm was used for both sensitisation and challenge. After application of the patch the patients were given a reminder note with the day and time when the patch had to be removed.

Sensitivity was assessed by measurement of responses to a series of four simultaneously applied challenge doses (Table 4.1). This was done 4-7 weeks after sensitisation in the majority of patients except three patients who were challenged 8 weeks, 9 weeks and 32 months after sensitisation due to time factors. The four challenge doses increased by a factor of $\sqrt{2}$ and were below the threshold for irritancy (Friedman, 1994 #320). The four challenge doses for each group were chosen based on the results of a small pilot study, which showed that transplant patients had no response at doses lower than 6.25μg. In non-immunosuppressed patients doses above 6.25μg elicited uncomfortably strong responses. The tests were applied on the inner arm for 6 hours (Rees et.al, 1990). Patients were given a reminder slip with the time of patch removal.

Measurements were done at baseline at, 48 hours and 96 hours. Erythema was assessed both clinically and objectively with the Erythema Meter (Dia-Stron Ltd., Andover, UK). The induration was assessed clinically and semi-quantitatively with the modified Harpenden calipers.

Table 4.1 Challenge doses of dinitrochlorobenzene

Group	2.2μg	3.125μg	4.325 μg	6.25 μg	8.75 μg	12.5 μg	17.7μg
Gr.1				**Yes**	**Yes**	**Yes**	**Yes**
Gr.2				**Yes**	**Yes**	**Yes**	**Yes**
Gr.3	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Gr.4	**Yes**	**Yes**	**Yes**	**Yes**			
Gr.5	**Yes**	**Yes**	**Yes**	**Yes**			

Gr.1-transplant patients with SCC; Gr.2-transplant patients without SCC; Gr.3-controls age, sex-matched with groups 1 and 2; Gr.4-Non-immunosuppressed patients SCC; Gr.5-Normal controls age and sex-matched with group

B. Recall antigens

The ability to mount a delayed-type hypersensitivity response to seven recall antigens given intradermally was assessed in all groups of patients. The seven common recall antigens are tetanus toxoid, diphtheria toxoid, streptococcus antigen, tuberculin, candida, trichophyton and proteus. A glycerine test was used as a negative control. The antigens and the glycerine control are available in a commercial test system, the Multitest CMI kit (Pasteur-Merieux, Lyon, France). The test was applied on the forearm and the response was assessed 48 hours later by measurement of the indurated area. If the reaction site was not circular two diameters at 90 degrees were measured and the sum divided by two. A reaction was positive if the diameter was 2mm or more. The score was zero if there was erythema but no induration. The sum of the diameters of the response was recorded as the patient's score. Normal men have sum scores of 10 or more and normal women have sum scores of 5 or more (Knicker *et al.*, 1984).

C. Contact allergens

A panel of common environmental allergens to which a proportion of the population reacts was tested by standard patch testing techniques. The tests are known to give reproducible results (Memon & Friedman, 1996) and aimed to assess the efferent arm of the immune response. The contact allergens were 5% (w/w) nickel (present in jewellery, zips, studs and buckles), 0.5% (w/w) potassium dichromate (cement), 8% (w/w) fragrance mix and 25% (w/w) balsam of Peru (perfumes, after-shaves, toiletries and cosmetics) and 20% (w/w) colophony (sticking plasters and adhesives) (Chromotechnique Diagnostics). White soft paraffin was used as a vehicle and a test with paraffin alone was used as a negative control.

The contact allergens were applied on strips of 8mm diameter Finn chambers (Epitest Ltd, Helsinki, Finland) on the upper back. The upper back has been recommended as the optimum site for patch testing (Memon & Friedman, 1996). A disposable syringe was used as a container and a 5mm length of test substance was delivered. Each strip of allergens was weighed prior to application to ensure that similar quantities of allergens were used for each patient. The patch tests were left on for 48 hours and the response was assessed at 48 hours and 96 hours.

Responses were graded, as recommended by the International Contact Dermatitis Research Group (Wilkinson *et al.*, 1970), as follows:

?+	doubtful reaction; faint erythema only
+	weak positive reaction; erythema, infiltration, possible papules
++	strong positive reaction; erythema, infiltration, papules, vesicles
+++	extreme positive reaction; intense erythema and infiltration and coalescing vesicles
IR	irritant reaction
NT	not tested

In addition erythema was measured with the Erythema Meter and transepidermal water loss was measured with the TEWL meter.

D. Irritants

Inflammatory responses to Dithranol (Aldrich Chemicals Ltd.) and Sodium Lauryl Sulphate (Aldrich Chemicals Ltd.) were tested in all groups of patients.

Dithranol is an aromatic compound widely used in the treatment of psoriasis, which has the unwanted side effect of causing considerable cutaneous irritancy. Dithranol in white soft paraffin was used in the following concentrations (w/w): 0.0025%, 0.005%, 0.01%. A disposable syringe was used as a container and a 5mm length of test substance was delivered on 8mm Finn Chamber strips (Epitest Ltd, Helsinki, Finland). The strip with the three test doses of dithranol and white soft paraffin (negative control) was applied on the upper back. Erythema, transepidermal water loss, skin hydration and clinical readings were done at baseline, 48 hours and 96 hours.

Clinical responses were graded as follows:

0	no reaction
½	weak erythema
1	moderate erythema
2	strong erythema
3	very strong erythema with oedema

Sodium lauryl sulphate (SLS) is an anionic surfactant added to cosmetics and toiletries, which has been used experimentally in studies of irritancy. SLS (purity over 90%) in sterile water was used in the following w/v concentrations: 0.0625%, 0.125%, 0.25%, 0.5%, 1% (for all groups); 2% (only groups 1 and 2). Fifty microlitres of test solution, the volume used for testing, are sufficient to saturate the filter paper disk applied on an 11mm Finn Chamber (de Boer & Bruynzeel, 1996). Sterile water was used as a negative control. Erythema, transepidermal water loss and skin hydration were assessed

at baseline, 48 hours and 96 hours. Clinical scoring was graded as recommended by the European Society of Contact Dermatitis (ESCD) presented in Table 4.2.

Table 4.2 ESCD guidelines on clinical scoring of acute SLS irritant reactions

Score	Qualification	Description
0	Negative	No rection
½	Doubtful	Very weak erythema and minute scaling
1	Weak	Weak erythema, slight oedema, slight scaling and/or slight roughness
2	Moderate	Moderate erythema, oedema, scaling and/ or roughness, minor degrees of erosions, vesicles, crusting and / or fissuring
3	Strong	Marked degrees of: erythema, oedema, scaling, roughness, erosions, vesicles, bullae, crusting and/ or fissuring
4	Very strong	As 3, with necrotic areas

4.2.2.2 Instruments: principles and methodology of measurement

All measurements were done in a temperature and humidity-monitored environment (MPM 500e unit from Solomat I.M., Devon, UK). Measurements were performed at the same time of the day on each of the three days to avoid physiological variations associated with the diurnal cycle. The subjects were allowed to acclimatise at room temperature for at least 15 minutes before the measurements were started and the position of the subjects was standardised during the measurements. All test sites were marked before application of the test substance.

A. Erythema Meter

The principle of operation of the Erythema Meter (Dia-Stron Ltd., Andover, UK) is based upon the technique of reflectance spectrophotometry and utilises the fact that haemoglobin present in the blood vessels in the upper dermis is

the main cutaneous chromophore of green light (Diffey *et al.*, 1984). When the blood content of the subpapillary plexus increases, resulting in erythema, there is an increase in the amount of absorbed and a decrease in the amount of reflected green light. In contrast, the amount of red light absorbed or reflected changes very little with vasodilation. By comparing the amount of reflected red and green light, an index is obtained which depends mainly on the blood content of the superficial dermis. The erythema index is expressed as

$$\text{Erythema index} = \log (1/R_g) - \log (1/ R_r),$$

where R_g and R_r are the reflectance for green and red light.

The white light from a bulb is focused on the aperture of one branch of a trifurcated fibre optic cable. Light re-emitted from the skin passes up the other two branches. One of the two branches is optically coupled to a green interference filter and a photodiode and the other is optically coupled to a red interference filter and photodiode. The photocurrents from both photodiodes are amplified and form the two inputs to the log ratio module. The output is displayed as the erythema index. The instrument is quick and easy to handle, may be used in normal room lighting and has been shown to be more sensitive than the eye (Farr & Diffey, 1984). Five measurements were taken within each test area and the mean of the five readings was used as the erythema index for the test site.

B. Trans epidermal water loss (TEWL) meter

The TEWL meter of the Dermalab unit (Cortex Technology, Hadsund, Denmark) was used to assess levels of transepidemal water loss.

Transepidermal water loss refers to the rate at which water migrates from the viable dermal tissues through the layers of the stratum corneum to the external environment (Berardesca & Maibach, 1988). The principles underlying TEWL measurement were reviewed by Pinnagoda & Tupker (Pinnagoda & Tupker,

1995). In the absence of forced convections, the human skin surface is surrounded by a water vapour boundary layer, which constitutes the transition zone for transportation of moisture and heat from the body to the ambient air. Considering the skin surface as a water-permeable surface, the process of water exchange through this zone can be expressed in terms of its vapour pressure gradient. This gradient is approximately constant in the absence of forced convection and under steady-state conditions and is proportional to the amount of water vapour passing through this zone per unit time and area by the evaporation from the skin surface. The vapour pressure gradient expressed as g/m^2h, is computed from the difference between the vapour pressures measured at two different fixed heights situated perpendicularly above the skin surface and within the zone of diffusion.

The measuring probe consists of two sensor units positioned at different distances from the skin inside a small cylinder, which forms the head of the probe and protects the sensors from air turbulence. Each sensor is composed of a humidity transducer and a thermistor. The vapour pressure at each fixed height of measurement above the skin is calculated from the formula

$$P=RH * p_{sat}$$

Where p is the water vapour pressure, RH is the relative humidity and the p_{sat} is the saturation vapour pressure (Pa). The calculated difference in the vapour pressure at the two fixed heights of measurement is the estimated vapour pressure gradient of the boundary layer. From this gradient the evaporative TEWL value is calculated. The reading is taken over 1 minute or the minimum period of time taken for the standard deviation to reach the lowest value.

The recommendations of the European Society of Contact Dermatitis are that before measurements the area tested should be allowed to acclimatise to ambient temperature for 15-30 minutes, the ambient temperature should be

maintained between 20-22°C and relative humidity should be regulated to about 40% (Pinnagoda, 1990 #324).

C. Corneometer

The superficial portion of the stratum corneum remains supple and flexible, as long as its water-holding capacity is intact. The Corneometer of the Dermalab unit (Cortex Technology, Denmark) evaluates the hydration state of the skin surface by measurement of skin impedance. Impedance (Z) the total electrical opposition to the flow of an alternating current, depends on two components, resistance (R) and capacitance (C). The relationship between these variables can be formulated as

$$Z=[R^2+(1/2\pi fC)^2]^{1/2},$$

where f is the frequency of an applied alternating current.

The probe consists of two concentric electrodes separated by a dielectric. When the probe is placed on the skin there is a rapid initial increase in both conductance and capacitance, followed by a gradual increase if the contact is maintained. The level of the initial increase represents the hydration state of the skin surface at the time of application of the probe and the later slow increase is due to accumulation of water beneath the probe from insensible percutaneous water loss (Tagami, 1995).

4.2.2.3 Statistical analysis

Two types of statistical tests, analysis of variance and linear mixed effects model, were used depending on whether a subject was considered to be measured more than once in the specific comparison (Montgomery, 1984; Vonesh & Cinchilli, 1997). For example the analysis of variance was used to test differences by group in variable Y given a particular test site and a particular time. The linear mixed effects model was used to test differences within the same group across the three time points when measurements were taken. The linear mixed effects model is equivalent to the analysis of variance if an individual appears only once in the analysis. Areas under the curve were computed by joining with straight lines the points of response y1 to y4 to the

doses x1 to x4. The area for each group was computed using the formula A=a1*y1+a2*y2+a3*y3+a4*y4 where a1=(x2-x1)/2, a2+(x3-x1)/2, a3+x4-x2)/2, a4+(x4-x3)/2. The standard deviation for the area A was the square root of the variance SD(A)=√Var(A).

4.3 Results

Nineteen patients were recruited in each of the groups 1, 2 and 3. Seventeen patients were recruited in each of the groups 4 and 5. The demographic details are presented in Table 4.3.

Table 4.3 Demographic data

Variable	Gr. 1	Gr. 2	Gr. 3	Gr. 4	Gr. 5
Number of patients	19	19	19	17	17
Male/Female	15/4	15/4	15/4	13/4	13/4
Mean age at testing	60.8	57.9	61.2	65.7	65.8
Males	60.8	56.9	60.9	63.8	63.2
Females	60.8	61.8	62.3	71.8	74.0
Mean age at transplantation	45.5	41.4	NA	NA	NA
Males	45.5	40.9	NA	NA	NA
Females	45.5	44.3	NA	NA	NA
Mean cumulative dose (g)					
Cyclosporine	626.1	530.3	NA	NA	NA
Azathioprine	568.2	596.5	NA	NA	NA
Prednisolone	43.5	39.2	NA	NA	NA
Methylprednisolone	4.4	4.2	NA	NA	NA
ATG (standard doses)	0.6	0	NA	NA	NA

NA= not applicable; Gr.1-RTRs with SCCs; Gr.2-RTRs without SCCs, Gr.3-controls for Gr.1 and 2; Gr.4-non-immunosuppressed patients with SCCs; Gr.5-controls for Gr.4.

Renal transplant recipients with skin cancer had received significantly more cyclosporine ($p=0.02$) and antithymocyte globulin (ATG) ($p<0.001$) than renal transplant recipients without skin cancer. There were no significant

differences in the doses of prednisolone and azathioprine between renal transplant recipients with and without skin cancer.

4.3.1 DNCB

The response was assessed as the change in erythema (ΔE) and skin fold thickness (ΔSFT) for each test site at 48 hours and 96 hours. ΔE and ΔSFT were computed by subtracting the value of the baseline reading from the reading at 48 and 96 hours. The following comparisons were made:

- group 1 versus group 2
- group 1 versus group 3
- group 2 versus group 3
- group 4 versus group 5
- group 1versus group 4

4.3.1.1 Erythema

The mean erythema readings are presented in Appendix A.18 *DNCB: Mean erythema reading*. The mean variations in erythema indices (ΔE) are presented in graphical form in Figures 4.2 and 4.3 and in tabular form in Table 4.4. The results of the linear mixed effects model analyses are presented in Appendix A.19 *DNCB: Results of linear mixed effects model analysis for ΔE*.

The erythema increased with the dose. Most of the variation occurred within the first 48 hours but with few exceptions readings continued to increase between 48 and 96 hours. For all doses, renal transplant patients with SCCs had lower responses than transplant patients without SCCs although as the dose increased the difference in response between the two groups narrowed. The difference reached statistical significance for 8.75μg at 48 hours and was marginally significant for 12.5μg at both 48 and 96 hours.

One dose, 6.25μg, was tested in all groups. Both groups of transplant patients had significantly lower responses than their control group. Non-immunosuppressed patients with SCC had lower erythema responses than

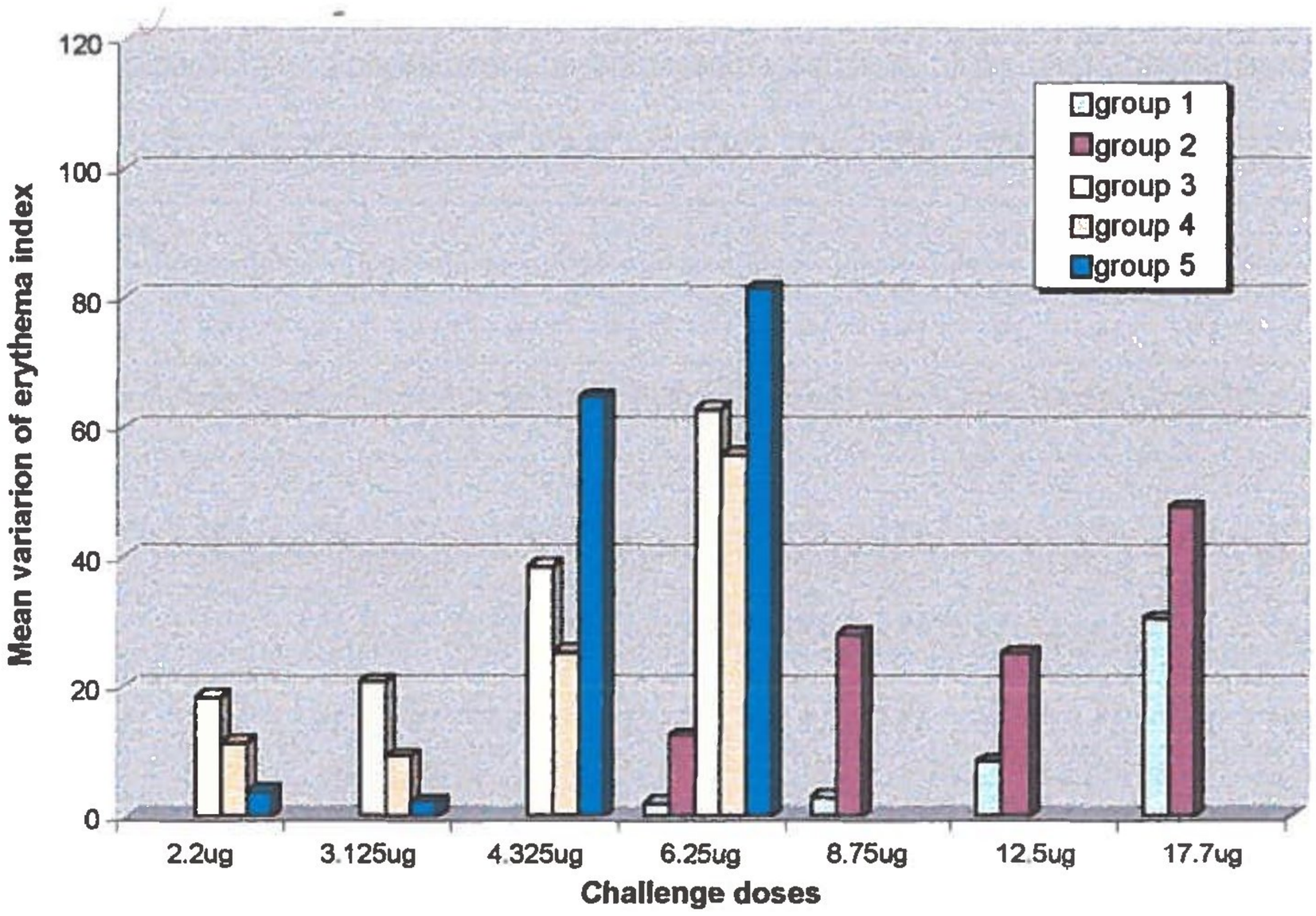


Fig.4.2 Mean variation in erythema index at 48

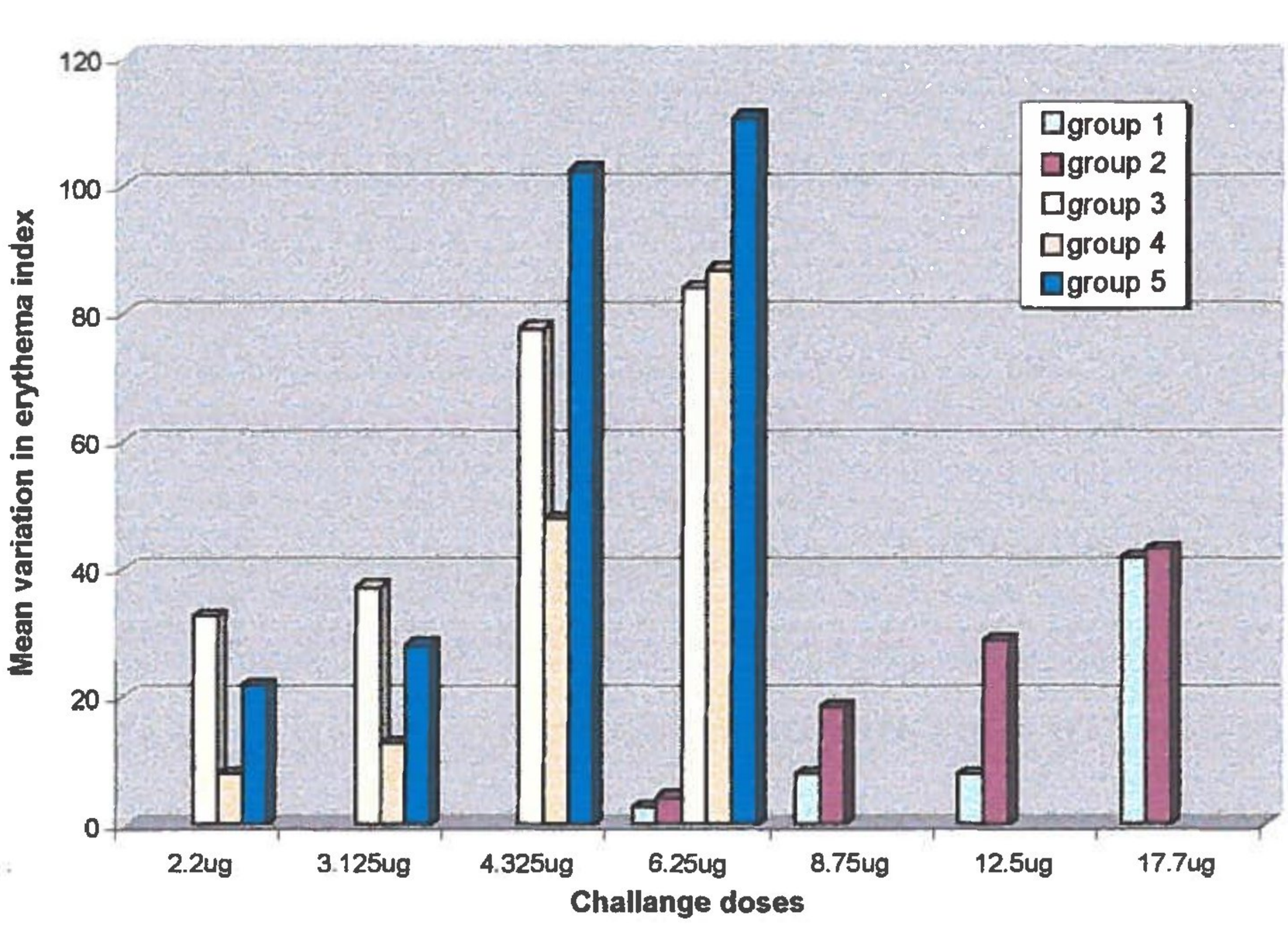


Fig. 4.3 Mean variation of erythema index at 96 hours

Gr.1- RTRs with SCCs; Gr.2-RTRs without SCCs; Gr.3-controls for Gr.1 and Gr.2; Gr.4-non-immunosuppressed patients with SCCs; Gr.5-controls for Gr.4.

their controls for all doses and times except 2.2μg and 3.125μg, at 48 hours. However for the same doses at 96 hours, non-immunosuppressed patients with SCC had lower responses than normal controls. The difference in erythema responses between these two groups reached statistical significance at 4.325μg.

Table 4.4 Mean variation in erythema (ΔE) at DNCB challenge sites

Site	Time	Group1 Mean (SD)	Group 2 Mean (SD)	Group 3 Mean (SD)	Group 4 Mean (SD)	Group 5 Mean (SD)
C	48 h	-2.0 (16.4)	0.4 (13.4)	1.6 (9.7)	1.0 (10.0)	-0.2 (12.4)
	96 h	4.6 (17.2)	1.6 (17.6)	-0.3 (11.8)	3.9 (15.0)	-0.7 (13.4)
2.2μg	48 h	NA	NA	18.2 (34.9)	11.1 (21.0)	3.6 (12.9)
	96 h	NA	NA	32.6 (52.7)	7.7 (27.6)	21.8 (42.7)
3.125μg	48 h	NA	NA	20.7 (31.5)	9.2 (18.0)	2.2 (16.7)
	96 h	NA	NA	37.0 (42.5)	12.6 (23.2)	28.0 (45.6)
4.325μg	48 h	NA	NA	38.5 (50.4)	25.2 (38.1)	64.6 (61.5)
	96 h	NA	NA	77.5 (60.4)	47.6 (67.6)	102.2 (80.6)
6.25μg	48 h	1.9 (16.1)	12.2 (28.1)	62.7 (50.6)	55.7 (50.2)	81.3 (65.2)
	96 h	2.4 (21.4)	4.0 (27.9)	83.7 (51.3)	86.5 (77.1)	110.5 (72.4)
8.75μg	48 h	2.8 (24.0)	27.9 (38.6)	NA	NA	NA
	96 h	7.8 (30.0)	18.3 (42.5)	NA	NA	NA
12.5μg	48 h	8.3 (24.5)	25.1 (28.9)	NA	NA	NA
	96 h	7.8 (23.9)	28.5 (33.6)	NA	NA	NA
17.7μg	48 h	30.3 (43.2)	47.6 (34.5)	NA	NA	NA
	96 h	41.7 (65.2)	43.0 (41.7)	NA	NA	NA

NA=not applicable; Gr.1-RTRs with SCCs; Gr.2-RTRs without SCCs; Gr.3-controls for Gr.1 and Gr.2; Gr.4-non-immunosuppressed patients with SCCs; Gr5-controls for Gr.4.

Another way of assessing erythema responses is by computing the area under the response curve (AUC) for each of the five groups of patients, at 48 and 96 hours. This makes stronger the comparison between groups, which received the same challenge doses. The following results were obtained:

Group	Time	Mean area (SD)
Gr.1	48h	127.04 (175.11)
	96h	170.70 (222.89)
Gr.2	48h	338.52 (201.38)
	96h	301.52 (230.69)
Gr.3	48h	150.91 (99.77)
	96h	256.04 (118.22)
Gr.4	48h	107.89 (79.61)
	96h	174.58 (132.03)
Gr.5	48h	183.19 (116.28)
	96h	305.87 (153.14)

Gr.1-RTRs with SCCs, Gr.2-RTRs without SCCs; Gr.3-controls for Gr.1 and Gr.2; Gr.4-non-immunosuppressed patients with SCCs; Gr.5-controls for Gr.4.

The AUC for the transplant patients with SCC was smaller than for transplant patients without SCC both at 48 and 96 hours. Non-immunosuppressed patients with SCC had lower responses than their control group both at 48 and 96 hours. The responses of the transplant control group were slightly lower than of the non-immunosuppressed control group.

4.3.1.2 Skin fold thickness

The results of the skin fold measurement are presented in Table 4.5, as the variations at 48 and 96 hours compared to the baseline (ΔSFT). The results of the mixed effects model analysis of ΔSFT are presented in Appendix A.20 *DNCB: Linear mixed effects model analysis of ΔSFT*. Skin fold thickness as a measure of oedema increased with the dose of DNCB for all groups. Most of the increase occurred in the first 48 hours and with few exceptions skin fold thickness continued to increase until 96 hours. Renal transplant recipients with SCCs had less oedema in response to DNCB compared to renal transplant recipients without SCCs, except for the highest dose 17.7μg, where the results were similar. Both groups of renal transplant patients had significantly less oedema ($p<0.001$) than their normal controls. The level

reached by renal transplant patients at 17.7μg was comparable to the level reached by their normal controls at 3.125μg. Similarly, non-immunosuppressed patients with SCCs had lower oedema responses compared to their normal controls except for the lowest doses, 2.2μg and 3.125μg, at 48 hours, but none of these reached statistical significance. At 17.7μg the oedema response in renal transplant patients with SCCs was significantly less than in non-immunosuppressed patients with SCCs.

Table 4.5 Mean variation in skin fold thickness at DNCB challenge sites

Site	Time	Group 1 Mean (SD)	Group 2 Mean (SD)	Group 3 Mean (SD)	Group 4 Mean (SD)	Group 5 Mean (SD)
C	48 h	0.02 (0.1)	0 (0)	0.02 (0.1)	0.03 (0.1)	0.02 (0.1)
	96 h	0.02 (0.1)	0 (0)	0.01 (0.1)	0.03 (0.1)	0.02 (0.1)
2.2μg	48 h	NA	NA	0.12 (0.3)	0.05 (0.1)	0.05 (0.1)
	96 h	NA	NA	0.22 (0.4)	0.09 (0.4)	0.14 (0.2)
3.125μg	48 h	NA	NA	0.32 (0.6)	0.11 (0.3)	0.09 (0.2)
	96 h	NA	NA	0.54 (0.7)	0.11 (0.4)	0.36 (0.5)
4.325μg	48 h	NA	NA	0.47 (0.6)	0.23 (0.4)	0.48 (0.5)
	96 h	NA	NA	0.66 (0.6)	0.50 (0.6)	0.90 (0.8)
6.25μg	48 h	0.01(0.1)	0.01(0.1)	0.74 (0.7)	0.56 (0.6)	0.79 (1.0)
	96 h	0.03 (0.1)	0.02 (0.1)	1.13 (0.7)	0.76 (0.9)	1.21 (1.0)
8.75μg	48 h	0.01(0.1)	0.08 (0.2)	NA	NA	NA
	96 h	0.05 (0.1)	0.11 (0.3)	NA	NA	NA
12.5μg	48 h	0.04 (0.1)	0.15 (0.4)	NA	NA	NA
	96 h	0.09 (0.2)	0.19 (0.5)	NA	NA	NA
17.7μg	48 h	0.28 (0.3)	0.21 (0.3)	NA	NA	NA
	96 h	0.26 (0.4)	0.25 (0.4)	NA	NA	NA

4.3.1.3 Clinical erythema and oedema

The number of patients with clinically detectable erythema/ oedema in each of the groups and test sites is presented in Table 4.6. Groups 1, 2 and 3 had 19 patients each. Groups 4 and 5 had 17 patients each. The clinical response mirrored the quantitative measurements. Fewer renal transplant patients with

SCCs had both erythema and oedema responses compared with renal transplant patients without SCCs, except for the highest dose 17.7μg, where the results were similar. Fewer non-immunosuppressed patients with SCCs had responded to DNCB compared to their normal controls when tested with the lower doses: 2.2μg and 3.125μg. At higher doses (4.325μg and 6.25μg) the results were similar. The comparative response in the five groups is best demonstrated at 6.25μg (see Fig.4.4).

Table 4.6 DNCB - Clinically detectable erythema/ oedema

Site	Time	Gr.1*	Gr.2*	Gr.3*	Gr.4*	Gr.5*
2.2μg	48 h	NA	NA	4/2	2/2	3/1
	96 h	NA	NA	6/5	2/2	6/5
3.125μg	48 h	NA	NA	6/6	3/1	6/6
	96 h	NA	NA	10/9	4/4	8/7
4.325μg	48 h	NA	NA	12/9	9/6	12/10
	96 h	NA	NA	13/13	10/9	14/11
6.25μg	48 h	1/0	4/2	15/15	11/11	14/12
	96 h	1/0	4/2	15/15	10/10	16/16
8.75μg	48 h	4/0	8/3	NA	NA	NA
	96 h	4/1	6/2	NA	NA	NA
12.5μg	48 h	6/0	11/2	NA	NA	NA
	96 h	5/1	9/6	NA	NA	NA
17.7μg	48 h	13/5	14/4	NA	NA	NA
	96 h	9/4	11/7	NA	NA	NA

*Figures represent number of patients with erythema/ oedema; Gr.1-RTRs with SCCs; Gr.2-RTRs without SCCs; Gr.3-controls for Gr.1 and Gr.2; Gr.4-non-immunosuppressed patients with SCCs; Gr.5-controls for Gr.4.

4.3.2 Recall antigens

The recall antigens tested were: tetanus, diphtheria, streptococcus (group C), tuberculin, candida albicans, trichophyton mentagrophytes and proteus mirabilis. The results are presented in Tables 4.7 and 4.8. None of the patients reacted to glycerine (negative control). There was one positive reaction (tuberculin) in the group of transplant patients with SCCs compared to four positive reactions in the transplant patients without SCCs and 39 positive

reactions in their matched normal controls. There were 27 positive responses in the group of non-immunosuppressed patients with SCC and only 23 positive responses in their matched control group.

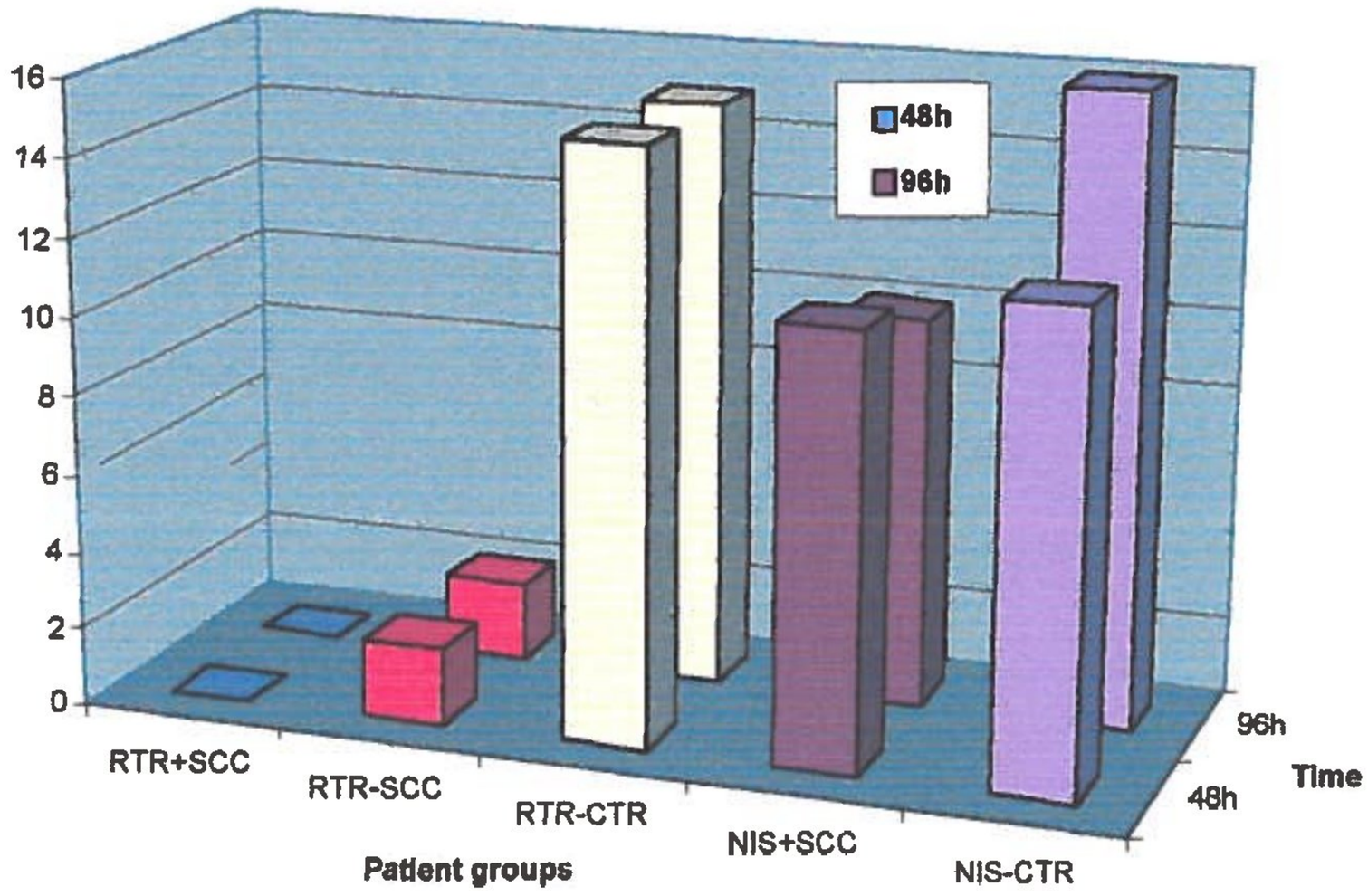


Fig. 4.4 Clinical erythema/oedema at 6.25μg

Table 4.7 Responses to recall antigens*

Group	No patients tested	Tetanus	Diphteria	Streptocc	Tuberculin	Candida	Trichophyton	Proteus
1	18	0	0	0	1(5.6)	0	0	0
2	18	2(11.1)	0	0	1(5.6)	0	0	1(5.6)
3	19	12(63.2)	2(10.5)	2(10.5)	8(42.1)	5(26.3)	6(31.6)	4(21.1)
4	17	7(41.2)	1(5.9)	2(11.8)	7(41.2)	3(17.6)	3(17.6)	4(23.5)
5	17	11(64.7)	1(5.9)	1(5.9)	5(24.9	2(11.8)	1(5.9)	1(5.9)

*figures represent number of patients with positive reactions; figures between the brackets represent percentages.

Sum scores for each group were computed by adding the diameters of the responses, expressed in centimetres to each of the recall antigens. A mean score for each group was calculated by dividing the sum score for the group by the number of patients tested.

Table 4.8 Sum scores (centimetres) of responses to recall antigens

Group	Tetanus	Diphtheria	Streptoc.	Tuberculin	Candida	Trichophyton	Proteus	Total score (mean score)
1	0	0	0	7	0	0	0	7 (0.4)
2	7	0	0	4	0	0	6	17 (0.9)
3	97	10	8	46	18	20	19	218 (11.5)
4	58	4	10	51	12	7	12	154 (9.1)
5	83	6	2	39	4	2	9	145 (8.5)

When assessing the diameter of the responses, transplant patients with SCCs had a lower score than transplant patients without SCCs and both groups had lower scores than the control group. The score for the non-immunosuppressed patients with SCC was slightly higher than for their matched controls.

4.3.3 Contact allergens

Few patients had positive reactions to contact allergens (Table 4.9). In the group of transplant patients with SCCs five patients had weak postive reactions to contact allergens: three patients to potassium dichromate, one patient to nickel and one patient to fragrance mix. No reactions occurred in group 2 and group 4 (where four patients had irritant reactions only). Two patients in each of the groups 3 and 5 had positive reactions. None of the patients reacted to colophony.

Table 4.9 Responses to contact allergens

Allergen	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**
Nickel	+ (1)	0	IR[1] (1)	IR (1)	+++ (1)
Potassium Dichromate	+ (3)	0	0	IR (2)	0
Fragrance Mix	+ (1)	0	IR (1)	0	++ (1)
Balsam of Peru	0	0	+ (1) ++ (1) IR (1)	IR (1)	0
Colophony	0	0	0	0	0

[1]IR=irritant reaction; figures between the brackets represent number of patients;Gr.1-RTRs with SCCs; Gr.2-RTRs without SCCs; Gr.3-controls for Gr1 and 2; Gr.4-NIS patients with SCCs; Gr.5-contrls for Gr.4.

4.3.4 Dithranol

Dithranol in white soft paraffin was used in the following concentrations (w/w): 0.0025%, 0.005%, 001%. In addition patients were tested to the vehicle agent, white soft paraffin.

4.3.4.1 Erythema

The mean erythema readings are presented in Appendix A.21 *Dithranol-Mean erythema readings*. Associated p values of the statistical tests are presented in Appendix A. 22 *Dithranol: Results of mixed effects model for ΔE.* The mean variation in erythema responses is presented graphically in Fig. 4.5 and 4.6. In all groups the response was maximal at 96 hours. For each of the dithranol concentrations erythema responses were higher in the groups of transplant patients with SCCs compared with the transplant patients without SCCs. The difference between the two groups was statistically significant for 0.0025% dithranol at 48 hours, and for all test sites at 96 hours. The response of the control group was intermediate between the two transplant groups. Non-immunosuppressed patients with SCC had only marginally higher erythema responses than their controls.

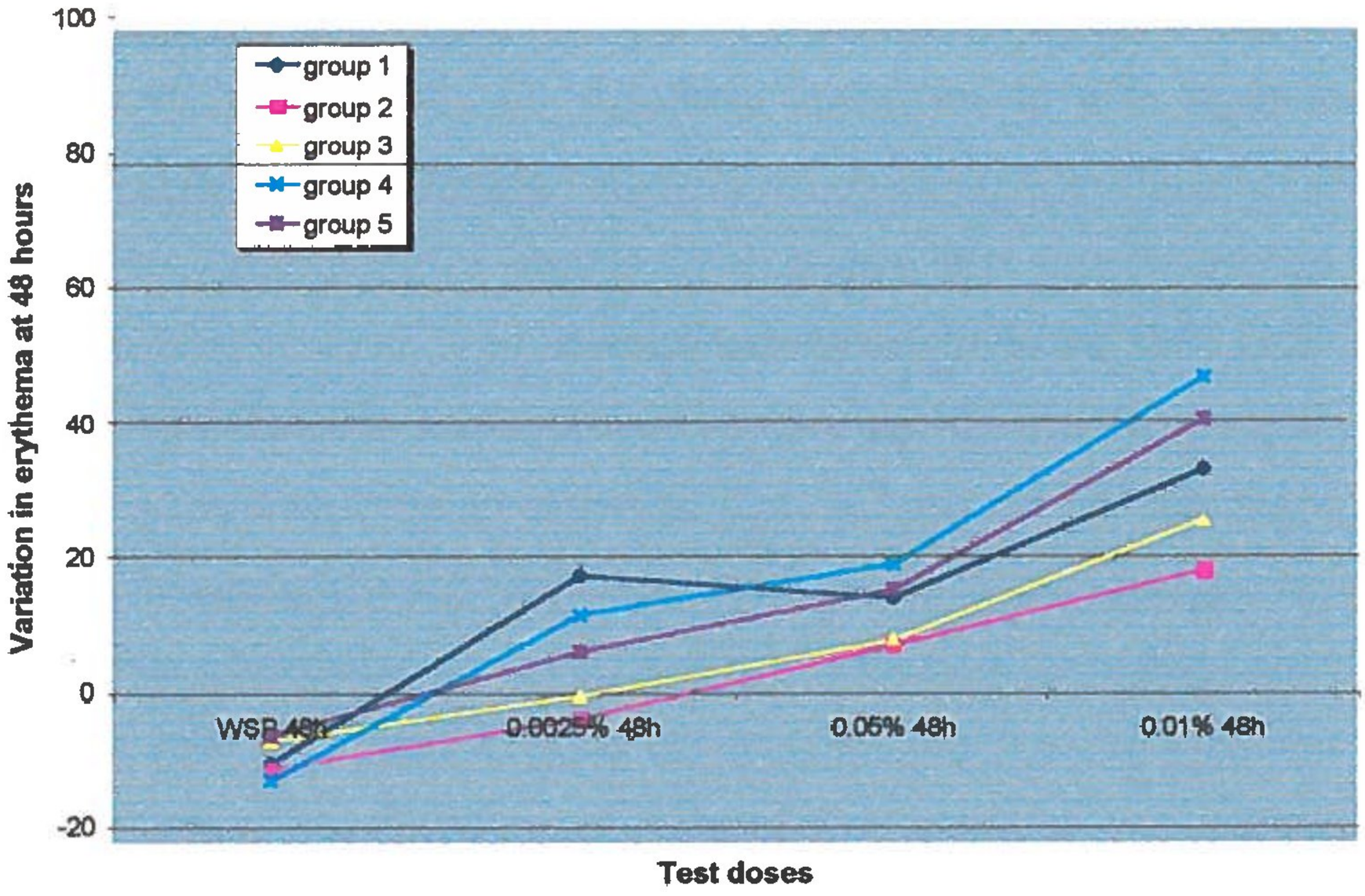


Fig.4.5 Dithranol – Erythema responses (ΔE) at 48 hours

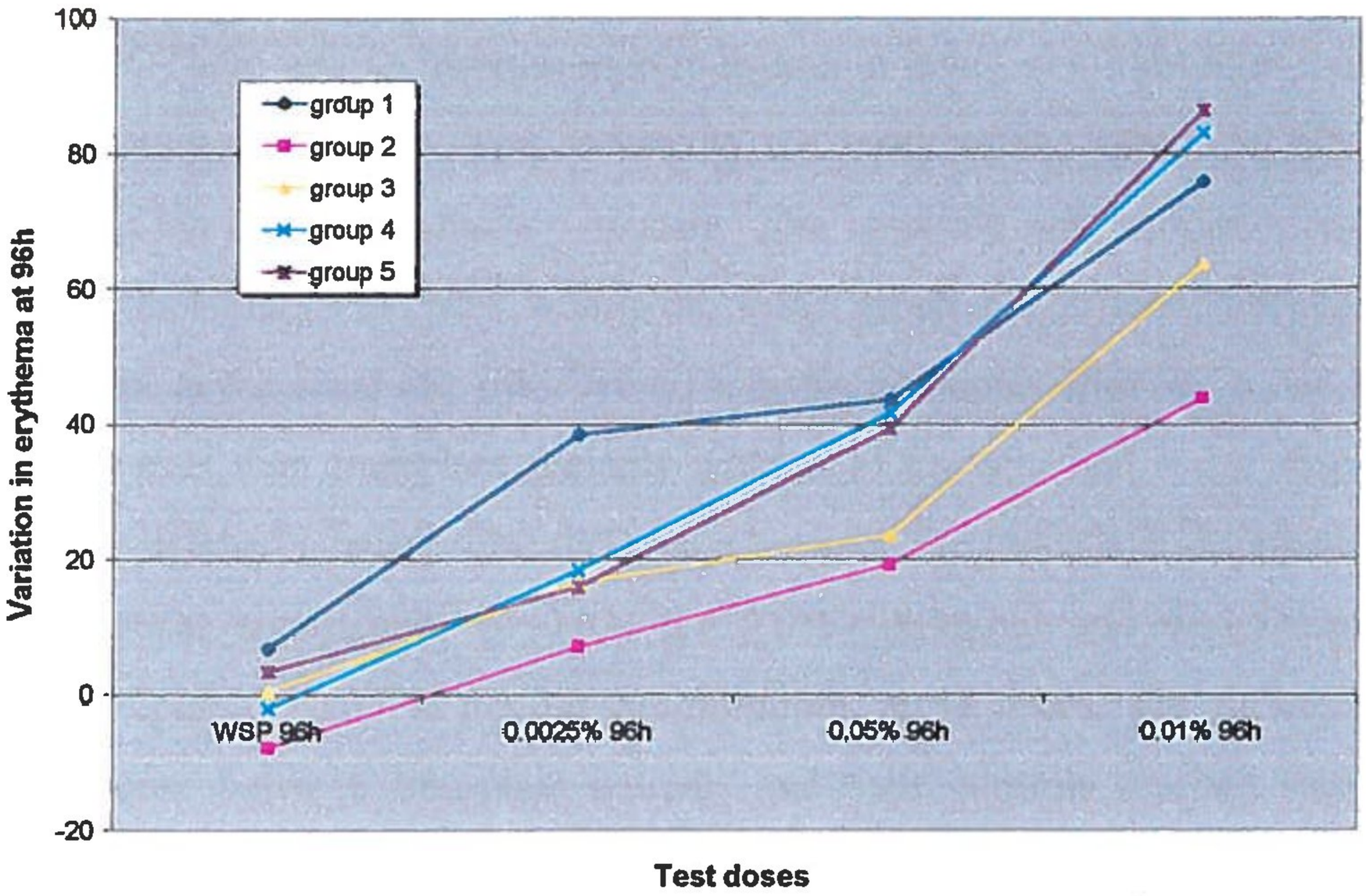


Fig. 4.6 Dithranol – Erythema responses (ΔE) at 96 hours

4.3.4.2 **Transepidermal water loss and skin hydration levels**

At baseline there was no statistically significant difference in TEWL levels although TEWL levels were slightly higher in the transplant patient groups. Similarly there was no significant difference in skin hydration levels (Appendix A.23 *Dithranol: Mean TEWL readings;* Appendix A.24 *Dithranol: Mean corneometer readings*). In all groups there was minimal increase in TEWL in response to all dithranol concentrations, which at 96 hours returned to near baseline levels. There was no significant difference in ΔTEWL between the pairs of groups tested. (2 vs.1, 3 vs.1, 3 vs.2, 4 vs.5, 4 vs.1) at 48 or 96 hours. The only exception was 0.0025% dithranol where, at 96 hours, group 4 had an increase of 0.2 g/m^2h from the baseline whereas group 5 had a decrease of 1.0g/m^2h from the baseline ($p=0.04$). It is likely that this isolated difference between the groups is due to experimental factors or chance alone.

The mean corneometer (CM) readings are presented in Appendix A.24 *Dithranol: Mean corneometer readings.* The results of the statistical analysis

are presented in Appendix A.25 *Dithranol: Mixed effects model analysis of ΔCM.* There was an increase in skin hydration levels at all test sites that was maximal at 48 hours. This is usually the result of the combined effect of occlusion and inflammatory exudate. The response was similar across the dithranol and WSP sites within the same group but differed between the groups at the same test site. Overall transplant patients with SCCs had lower responses than transplant patients without SCCs who had lower responses than matched controls. The difference between the transplant groups reached statistical significance (p=0.02) for 0.01% dithranol and was marginally significant (p=0.05%) for 0.005% dithranol, at 48 hours. The difference in response between transplant patients and their controls reached statistical significance for 0.01% dithranol. Non-immunosuppressed patients had lower responses than their matched controls for all the doses tested. The difference between the groups approached statistical significance (see Appendix A25 *Dithranol: Mixed effects model analysis of ΔCM).*

4.3.4.3 Clinical scoring

The mean clinical scores are presented in Table 4.10.

Table 4.10 Dithranol – clinical scores

Group	Time	Control	WSP	0.0025%	0.005%	0.01%
RTR+SCC	48h	0	0	0.23	0.42	0.89
	96h	0	0	0.34	0.56	1.09
RTR-SCC	48h	0	0	0.13	0.23	0.68
	96h	0	0	0.08	0.31	0.78
RTR – CTR	48h	0	0	0.16	0.45	1
	96h	0	0	0.18	0.42	0.95
NIS+SCC	48h	0	0.05	0.26	0.47	1.21
	96h	0	0	0.25	0.59	1.31
NIS – CTR	48h	0	0.03	0.2	0.41	1.03
	96h	0	0	0,18	0,44	1.32

The group of transplant patients with SCCs had slightly higher erythema responses compared with the group of transplant patients without SCCs in keeping with the objective erythema reading. One possible explanation is that dithrnol generates reactive oxygen species at molecualr level and patients with skin cancer have deficient antioxidant systems and therefore a more extensive reaction to dithranol. Nonimmunosuppressed patients with SCC had similar responses to their control group. In all groups there was a dose response increase in clinically detectable erythema.

4.3.5 Sodium Lauryl Sulphate (SLS)

Sodium lauryl sulphate in sterile water was used, for all groups, in the following w/v concentrations: 0.00625%. 0.125%, 0.25%, 0.5% and 1%. In addition renal transplant recipient groups were also tested with 2% SLS solution.

4.3.5.1 Erythema

The mean erythema readings are presented in Appendix A.26: *SLS: Mean erythema readings.* The associated p values of the statistical tests are presented in Appendix A.27 *SLS: Mixed effects model analysis of ΔE.* Erythema responses increased with the challenge dose in all groups and were maximal at 96 hours. The mean response in transplant patients with SCCs was lower than in transplant patients without SCCs, who had lower responses than the control groups but the difference in response between the transplant groups did not reach statistical significance. There was no statistically significant difference between transplant patients without SCCs and the control group. Erythema responses in non-immunosuppressed patients with SCC were similar to the control group. The results are presented graphically in Fig. 4.7 and Fig.4.8.

4.3.5.2 Transepidermal water loss and skin hydration levels

Mean values of TEWL are presented in Appendix A.28: *SLS: Mean TEWL readings.* The associated p-values of the statistical tests are presented in Appendix A.29 *SLS: Linear mixed effects model of ΔTEWL.* At baseline there was no significant difference in TEWL levels or skin hydration levels between

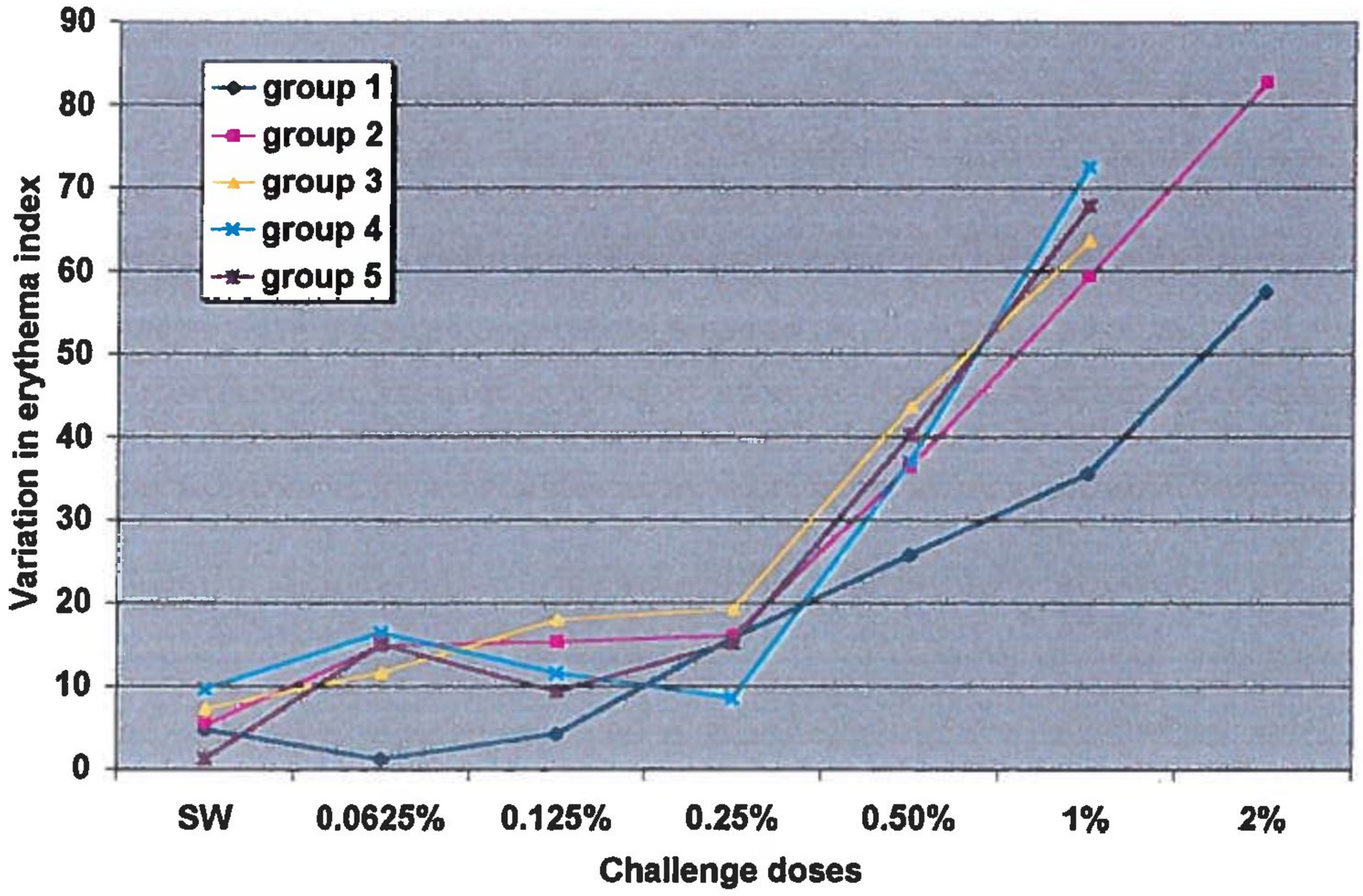


Fig. 4.7 SLS – erythema responses at 48h

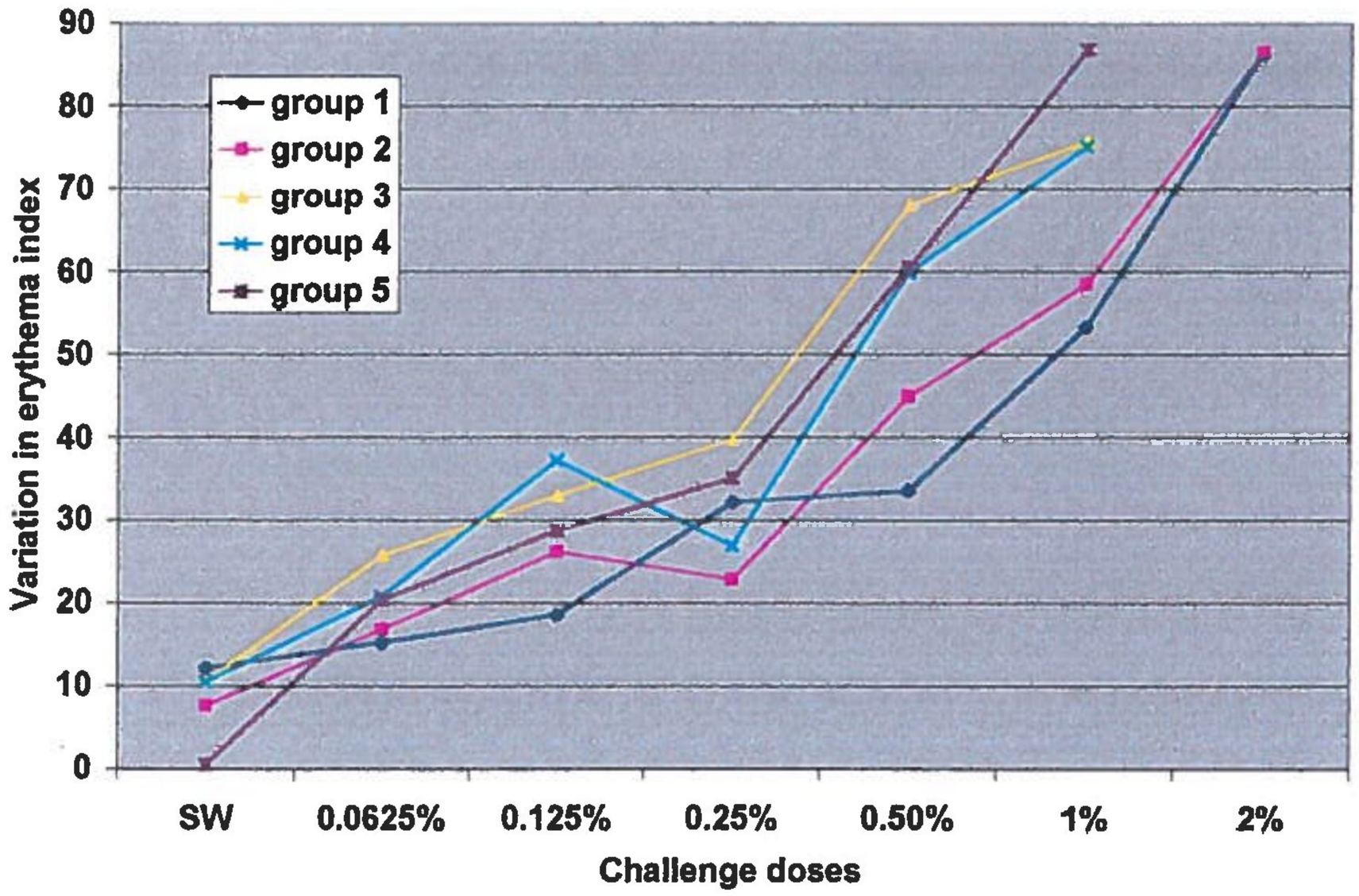


Fig. 4.8 SLS – erythema responses at 96 hours

the pairs of groups tested. Similar to measurements for dithranol, transplant patients with SCCs had slightly higher baseline levels of TEWL compared to the other groups. Overall the increase in TEWL was maximal at 48 hours followed by a reduction in response after this time. Across the groups there was a dose response increase in TEWL, more marked from 0.25% upwards(Fig.4.9). Transplant patients with SCCs had slightly lower responses compared with transplant patients without SCCs ($p=0.05$ for 0.5% at 48 hours; $p=0.006$ for 1% at 48 hours). The difference between the two groups was highest for 1% SLS. At 2% the response in transplant patients with SCCs was closer to the response in transplant patients without SCCs. At 1% the response was significantly lower in each of the transplant groups compared with controls. Non-immunosuppressed patients with SCCs had similar responses to their control group. For all the doses tested the response was lower in the immunosuppressed patients with SCCs compared to the non-immunosuppressed patients with SCC.

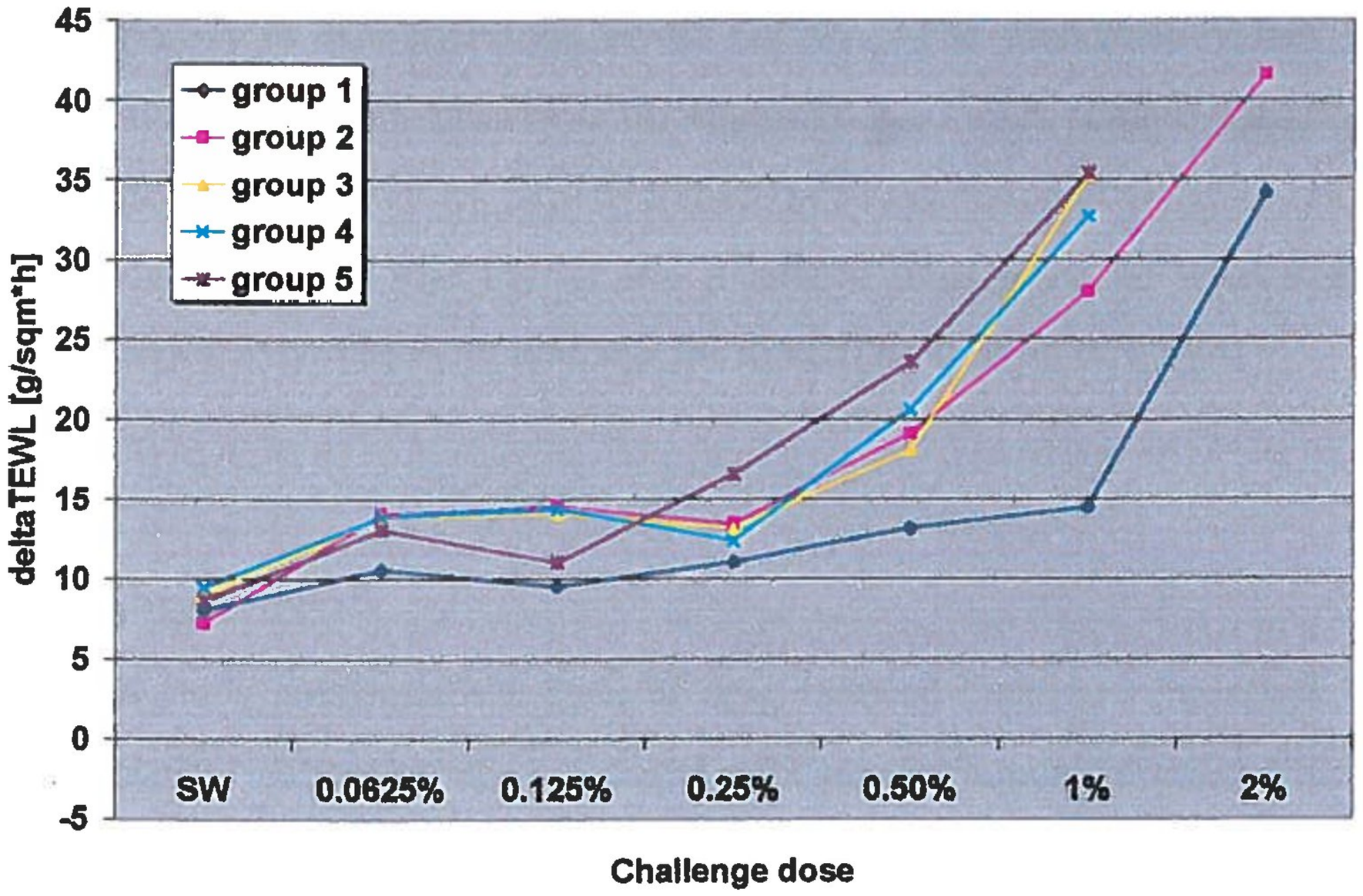


Fig. 4.9 SLS – Transepidermal water loss response at 48 hours

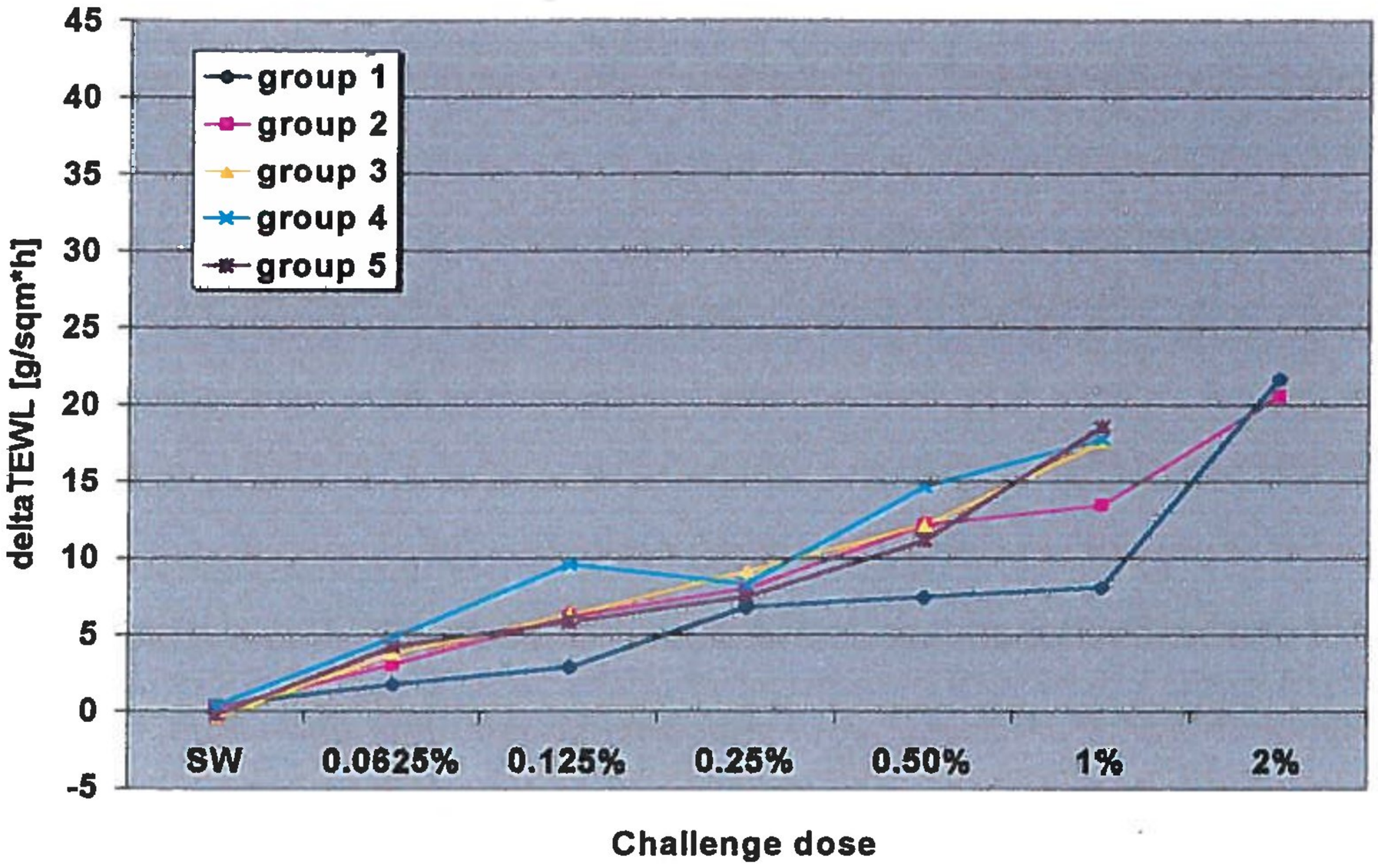


Fig.4.10 SLS – Transepidermal water loss response at 96 hours

Mean values of corneometer readings are presented in Appendix A.30 *SLS: Variation in skin hydration levels (ΔCM).* The associated values of the statistical tests are presented in Appendix A.31 *SLS: Linear mixed effects model analysis of ΔCM.* Skin hydration levels decreased at 48 hours as a result of disrupting the skin barrier and increased transepidermal water loss. The variation in corneometer readings mirrored the variation in TEWL.

4.4 DISCUSSION

This study confirmed the state of drug induced immune suppression in the transplant patient groups compared with non-immunosuppressed patients. It also investigated the differences in the degree of immune-responsiveness between transplant patients with and without skin cancer and non-immunosuppressed patients with skin cancer. For the purpose of this study we selected patients with squamous cell carcinoma (SCC), which is the most prevalent type of skin cancer in transplant patients.

Overall transplant patients appear to have lower delayed-type hypersensitivity responses, as reflected by the DNCB and recall antigen testing, than non-immunosuppressed patients with or without SCC. Testing with DNCB showed a dose dependent response in all groups. Most of the increase occurred in the first 48 hours but both erythema and skin fold thickness continued to increase up to 96 hours. Renal transplant patients with skin cancer had lower erythema responses than renal transplant patients without skin cancer for the same challenge doses. The difference approached statistical significance at 12.5μg. Both groups of transplant patients had significantly lower responses than their matched controls. Erythema responses in the renal transplant patients without SCCs at 6.25μg were comparable with the response achieved by normal controls at 2.2μg.

The area under the response curve for the renal transplant patients with skin cancer was about half of that of renal transplant patients without skin cancer. It is not possible to directly compare the AUC between renal transplant groups and their control group as the challenge doses used were different, except for 6.25μg. Non-immunosuppressed patients with SCC had a smaller AUC response than their control group. The difference between the two groups reached statistical significance at 4.325μg.

Skin fold measurement as an objective attempt to evaluate oedema showed a significantly lower change in SFT in the transplant groups compared with the normal controls or non-immunosuppressed patients with SCC. The increase in SFT was lower in the transplant patients with SCCs than in the transplant patients without SCCs but as in the case of erythema the results became more similar for the highest challenge dose (17.7μg). Clinical assessment of erythema/ oedema confirmed qualitatively the results of erythema and skin fold measurement. Only 26% of the transplant patients with skin cancer had an erythema / oedema response to the highest challenge dose of DNCB (17.7μg) compared with 37% transplant patients without skin cancer. In the control group 80% of the patients had an erythema/ oedema response to their

highest challenge dose (6.25μg), which was the lowest challenge dose for the transplant groups. With higher DNCB challenge doses and an increase in the antigenic stimulus the difference between the transplant groups narrowed. Both groups of transplant patients had significantly lower responses than their control group. Transplant patients with SCCs had significantly lower responses for DNCB than non-immunosuppressed patients with SCC. In turn non-immunosuppressed patients had lower erythema and oedema responses than their control group. It is likely that given a larger sample size the difference between the groups would have been larger and the statistics stronger. However we were limited in the selection process by the number of patients who fulfilled the criteria and were able to spare time for the study.

Very few transplant patients had positive reactions to recall antigens (see Table 4.7). Only two transplant patients without SCCs had a response to tetanus antigen compared to 11 and 12 patients in the control groups. Only one patient in each of the transplant groups reacted to tuberculin compared with 8 patients in the control groups. All patients would have been immunised for tuberculin and protection should be life long. The difference between the transplant groups and their matched controls became more evident when comparing the mean diameter of all responses (0.4mm for transplant patients with SCCs, 0.9mm for transplant patients without SCCs and 11.5mm for the control group). None of the transplant patients reacted to diphteria, streptococcus, candida or trichophyton. Non-immunosuppressed patients with SCC had responses similar to their control group.

Testing to contact allergens brought very little interpretable information due to the low number of responders.

Non-specific inflammatory responses to dithranol were higher in the group of transplant patients with SCCs than in the group of transplant patients without SCCs. This result contrasts with the results of the other tests and may be due to a specific property of dithranol or experimental design factors (site of test).

At baseline, TEWL levels were highest in the group of transplant patients with SCCs followed by transplant patients without SCCs, while normal controls had only half of the TEWL baseline levels of the first group. The skin of transplant patients appear to be losing more water and could therefore be more susceptible to the irritant action of dithranol and explain the higher responses in this group. There was no significant difference in responses to dithranol between the transplant and their control group or between non-immunosuppressed patients and their control group.

Inflammatory responses to SLS appeared to be slightly lower in the group of transplant patients with skin cancer compared to the transplant patients without skin cancer and the control group. At higher doses the results became more similar. Non-immunosuppressed patients with skin cancer had similar responses to their control group. Only two isolated comparisons were statistically significant. For the purpose of the study we regard the results as showing no statistically significant difference. All groups were able to show dose dependent responses to both dithranol and SLS.

In conclusion transplant patients with skin cancer have lower delayed type responses compared with transplant patients without skin cancer and require stronger antigenic stimuli to achieve a response. The most likely explanation is based upon the finding that transplant patients with skin cancer had received higher cumulative doses of cyclosporine compared with the transplant patients without skin cancer. Cyclosporine has been shown to inhibit the proliferation of CD4+ cells and the production of INFδ, both of which are required in the afferent and efferent stages of a delayed type response. It is therefore possible that there is a dose-dependent grading in the immunosuppressive effect of cyclosporine and this is reflected clinically in the form of skin cancers. It would be interesting to see whether patients with skin cancer have higher trough levels of cyclosporine or whether perhaps their lymphocytes are more sensitive to the effects of cyclosporine. Non-immunosuppressed patients with SCC had lower responses than their matched controls. None of these patients had ever received any treatment that would

have affected their immune responses and an alternative explanation for the reduced responses must be sought.

Non specific immune responses to irritants appeared to be similar between groups. Immune responses to irritants are non-specific and do not require sensitisation. Although T cells are present in the inflammatory infiltrate other cells like macrophages, natural killer cells and neutrophils, which are not affected by cyclosporine, accompany them.

With better knowledge of the metabolism of immunosuppressive drugs and optimal doses to prevent rejection it may be possible in the future to tailor doses according to individual needs and reduce side effects.

Chapter 5

HLA Types and TNF-α Polymorphisms and Skin Cancer Risk after Transplantation

5.1 Introduction

Epidemiological data suggests that environmental factors are the most important in the aetiology of cancer. However the response to such factors is determined in part by the genetic make-up of each individual. A number of genes have been explored in recent years in relation to the risk of skin cancer (reviewed in Chapter 2). In a renal transplant population the identification of genotypes at risk would allow us to give a better estimate of the risk of skin cancer prior to patients deciding in favour of renal transplantation and to select those patients who need more intense follow up after transplantation.

5.2 Human leukocyte antigens and the risk of skin cancer

5.2.1 Background

The identification by T cells of cells infected with intracellular pathogens depends on presentation of antigenic peptides on the surface of the cells by specialised surface glycoproteins. These glycoproteins are encoded in a large cluster of genes, which was first identified by their potent effects on the immune response to transplanted tissues. The gene complex is called the major histocompatibility complex (MHC) and the peptide binding

glycoproteins are called MHC molecules. In humans the MHC complex is called the human leukocyte complex (HLA). The HLA complex is located on the short arm of chromosome 6 and contains at least 50 genes in humans.

There are two different classes of MHC molecules known as MHC class I and MHC class II, each of which deliver peptides from a different cellular compartment to the surface of the infected cells. MHC class I present peptides from pathogens in the cytosol to CD8 cytotoxic T cells. MHC class II molecules deliver peptides originating in the vesicular system to the cell surface, where they are recognised by CD4 T cells. MHC class I molecules are expressed on almost all cells. MHC class II molecules are normally found on the surface of B-lymphocytes, macrophages and dendritic cells. MHC is polygenic: in humans there are three class I α-chain genes, called HLA-A,-B and –C and also three pairs of HLA class II α- and β-chains genes, called HLA-DR,-DP and –DQ. Each of the genes encodes proteins with different ranges of peptide binding specificities. MHC is also highly polymorphic, each gene having multiple alleles. The combination of polygeny and polymorphism extends the range of peptides that can be presented to T cells by an individual (Janeway, 1996 #283). Each person has two alleles for each locus. The alleles are co-dominant and therefore both antigens are expressed independently on the same cell.

In *non-immunosuppressed patients* a positive association has been reported between HLA DR1 and the development of multiple basal cell carcinomas (Cerimele *et al.*, 1988; Czarnecki *et al.*, 1991; Czarnecki *et al.*, 1993). HLA DR1 has also been linked with the risk of non-melanoma skin cancer with a stronger association for SCC than BCC (Glover *et al.*, 1993). A high frequency of HLA DR4 has been found in patients with multiple BCCs and malignant melanoma (Czarnecki *et al.*, 1993). An association between HLA DR7 and the risk of multiple non-melanoma skin cancer has also been reported from Australia (Czarnecki *et al.*, 1994).

In *renal transplant recipients* a negative association between HLA A11 and the risk of both basal cell carcinoma and squamous cell carcinoma has been reported in Europe (Bouwes Bavinck *et al.*, 1990; Bouwes Bavinck *et al.*, 1991b; McGregor *et al.*, 1992). In an Australian transplant population HLA A11 was associated with an increased risk of skin cancer (Bouwes Bavinck *et al.*, 1991a).

HLA B27 was a risk factor for the development of non-melanoma skin cancer both in Europe (Bouwes Bavinck *et al.*, 1990; Bouwes Bavinck *et al.*, 1991b) and Australia (Bouwes Bavinck *et al.*, 1997; Czarnecki *et al.*, 1992). In the Australian population HLA B27 alone contributed to the association, while in the Dutch population squamous cell carcinoma and basal cell carcinoma contributed equally to the association.

5.2.2 Patients and methods

Each patient who received a transplant was HLA typed as part of the work up prior to transplantation. At later dates when patients attended for their annual review additional samples were taken and HLA type re-checked and updated to conform to the HLA types agreed at international workshops. The Tissue Typing Laboratory of the Renal Transplant Centre in Oxford provided data on HLA types. The patient cohort comprised 979 patients. Of these 187 patients had at least one histologically confirmed skin cancer (see Chapter 3). The PCR technique (PCR-SSP) was used to genotype the HLA complex under universal conditions (Bunce *et al.*, 1995). Genomic DNA extraction (Welsh & Bunce, 1999), PCR amplification and gel electrophoresis (Bunce *et al.*, 1995) were conducted as described previously.

Associations were assessed using contigency table analysis and the χ^2 test (with Yates' correction) or Fisher's exact tests were used as required. Bonferroni correction was applied to correct for multiple comparisons.

5.2.3 Results

HLA B17 was significantly associated with the risk of SCC ($p<0.001$ after correction for multiple comparisons) and HLA B27 was associated with the risk of BCC. No significant association was found for other HLA alleles described in the literature (HLA A11, HLA DR1, HLA DR4, HLA DR7).

5.3 TNF α POLYMORPHISMS AND THE RISK OF SKIN CANCER

5.3.1 Background

Tumour necrosis factor (TNF) was discovered by Loyd Old who noticed that the capacity to induce, in vivo and in vitro, the necrosis of some mouse tumours could be ascribed to a factor present in the blood after lipopolysaccharide (LPS) injection, which was called tumour necrosis factor (Old, 1985). There are two TNF molecules, TNF-α and TNF-β, which share 30% homology, have an identical action, share the same receptors and are encoded by immediately adjacent genes (Vassalli, 1992 #354).

The human TNF-α gene is located in class III region of the MHC, 250 kb centromeric of the class I HLA B locus and 850 kb telomeric of the class II HLA DR locus (Higuchi, 1998 #357). Alleles of the MHC region have been linked to the risk of skin cancer. Because of the effect of TNF-α on tumour cells and the gene location within the MHC it is important to establish the contribution of TNF locus to these associations. Furthermore keratinocyte derived TNF-α mediates UVB-triggered secretion of PGE2, expression of ICAM-1 and keratinocyte apoptosis (Takashima, 1996 #363).

Eleven polymorphic sites have been described in an around TNF (Brinkman *et al.*, 1996; D'Alfonso, 1996; D'Alfonso, 1994; Hamann *et al.*, 1995; Higuchi *et al.*, 1998; Wilson *et al.*, 1993; Zimmerman *et al.*, 1996). Polymorphisms of the 5' flanking region of the gene are responsible for transcriptional regulation of TNF-α production. One allele (TNF_{-308G}) has been linked with an increased

risk of cancer and in particular lymphoma (Chouchane *et al.*, 1997) and TNF_{376A} has been shown *in vitro* to have lower levels of LPS-induced gene expression (Knight *et al.*, 1999). We have therefore set to investigate the effect of some of the TNF-α alleles on the risk of skin cancer in renal transplant recipients.

5.3.2 Patients and methods

Patients

One hundred and nineteen cases were selected from renal transplant patients who had received a cadaveric renal transplant, who developed at least one skin malignancy while on immunosuppression and had a DNA sample available. One hundred and fifteen controls were selected from renal transplant recipients who had had received a cadaveric renal transplant that survived for at least five years and had a DNA sample available (kindly provided by the Tissue Typing Laboratory, Transplant Centre, Oxford). The number of cases and controls was calculated based on the frequency in the population of the alleles studied in order to detect an odds ratio of at least 4 with a power of 80%.

PCR primer design

The TNF sequence GenBank Z15026 was used to generate primers. Allele specific primers were designed (by the Tissue Typing Laboratory, Transplant Centre, Oxford) to amplify regions of the gene containing the polymorphic sites (Table 5.1). Some of the primers were combined to link polymorphic sites within TNF-α (Fanning, 1997 #370) [Table 5.2]. In addition control primers were added to each reaction mix to confirm adequate DNA amplification.

PCR preparation and cycling conditions

DNA was extracted from EDTA or tri-sodium citrate anti-coagulated peripheral blood using a modified salting-out method (Miller, 1988 #349). DNA was then amplified in 13μl reaction mixtures consisting of 67mM Tris

Trisbase ph 8.8; 16.6mM ammonium sulphate; 2mM magnesium chloride; 0.01%v/v Tween 20; 200mM of each dATP, dTTP, dGTP and dCTP; between 0.1 and 0.01μg DNA and 0.1875 units of Taq polymerase (Advanced Biotechnology, London, UK). Primer concentrations were optimised for each reaction. Reaction mixtures were dispensed under 10μl of mineral oil (Sigma, UK) in 96 well PCR plates (AB Gene, Epsom, UK). DNA samples were amplified in MJ Research PTC-200 thermal cyclers, and PCR plates were sealed and dipped in mineral oil to improve plate-to-block contact. Cycling parameters were as follows: 1 minute at 96°C followed by 4 cycles of 96°C for 20 seconds, 70°C for 45 seconds and 72° C for 25 seconds, followed by 20 cycles of 96°C for 25 seconds, 65°C for 60 seconds and 72°C for 30 seconds, followed by 3 cycles of 96°C for 30 seconds, 55°C for 60 seconds and 72°C for 90 seconds and another 5 minutes at 20°C. Following PCR, 5μl of loading

Table 5.1 TNF-α primers sequences

Primer identity	Sequence
1971	5'-CAA Agg AgA AgC TgA gAA gAT
1972	5'-CAA Agg AgA AgC TgA gAA gAC
1973	5'-CgA gTA Tgg ggA CCC CCC
1974	5'-gAg TAT ggg gAC CCC CA
1975	5'-TCA CAT ggC CCT gTC TTC g
1976	5'-CTC ACA Tgg CCC TgT CTT CA
1977	5'-CCT gCA TCC TgT CTg gAA g
1978	5'-TCC TgC ATC CTg TCT ggA AA
1986	5'-CCg ggA ATT CAC AgA CCC C
1987	5'-ggC Tgg gTg TgC CAA CAA C
1988	5'-AAg gAT Aag ggC TCA gAg Ag
2403	5'-ATA ggT TTT gAg ggg CAT gg
2404	5'-ATA ggT TTT gAg ggg CAT gA
2405	5'-gAA gAC CCC CCT Cgg AAT Cg
2406	5'-gAA gAC CCC CCT Cgg AAT CA
710	5'-gCA TCC CCg TCT TTC TCC AT
711	5'-gCA TCC CCg TCT TTC TCC AT

buffer, consisting of 0.25% Orange G, 30% v/v glycerol and 0.5x TBE (89mM Tris base, 89mM boric acid, 2mM EDTA, pH 8.0) was added to each reaction mix. PCR products were electrophoresed in 1.0% agarose gels containing 10μg/l ethidium bromide for 25 minutes at 200V in 0.5x TBE buffer, visulalised with UV illumination and photographed (Fig.5.1).

Table 5.2 Primer mixes

HLA allelic specificity	Primer mixes	Size of amplicon
TNF 1032 T	1971-1986	433kb
TNF 1032C	1972-1986	433kb
TNF 865 C	1973-1986	263kb
TNF 865 A	1974-1986	262kb
TNF 859C	1988-1975	270kb
TNF 859A	1988-1976	270kb
TNF 380g	1977-1987	396kb
TNF 380A	1978-1987	397kb
TNF 488g/238g/308g	710-2403	835kb
TNF 488g/238g/308A	710-2404	835kb
TNF 488g/238A/308g	710-2406	763kb
TNF 488A/238g/308g	711-2405	763kb

Statistical analysis

Associations were assessed using contigency table analysis and the χ^2 test (with Yates' correction) or Fisher's exact tests were used as required. Bonferroni correction was applied to correct for multiple comparisons. Linkage disequilibrium between TNF and HLA alleles was calculated 2x2 contigency tables and chi-square tests.

5.3.3 Results

Associations were investigated between TNF genotypes ($TNF_{-1032T/-1032C}$, $TNF_{-865C/-865A}$, $TNF_{-859C/-859A}$, $TNF_{-380G/-380A}$, $TNF_{-488G/-488A/-488GA}$, $TNF_{-238G/-238A/-238GA}$, $TNF_{-308G/-308A/-308GA}$) and the risk of any skin malignancy and more

$_{238GA}$, $TNF_{-308G/-308A/-308GA}$) and the risk of any skin malignancy and more specifically the risk of SCC, BCC or Bowen's disease after transplantation. Only one association was found between TNF_{-1032T} allele and the risk of BCC (p=0.03), which became non-significant after correction for multiple comparisons.

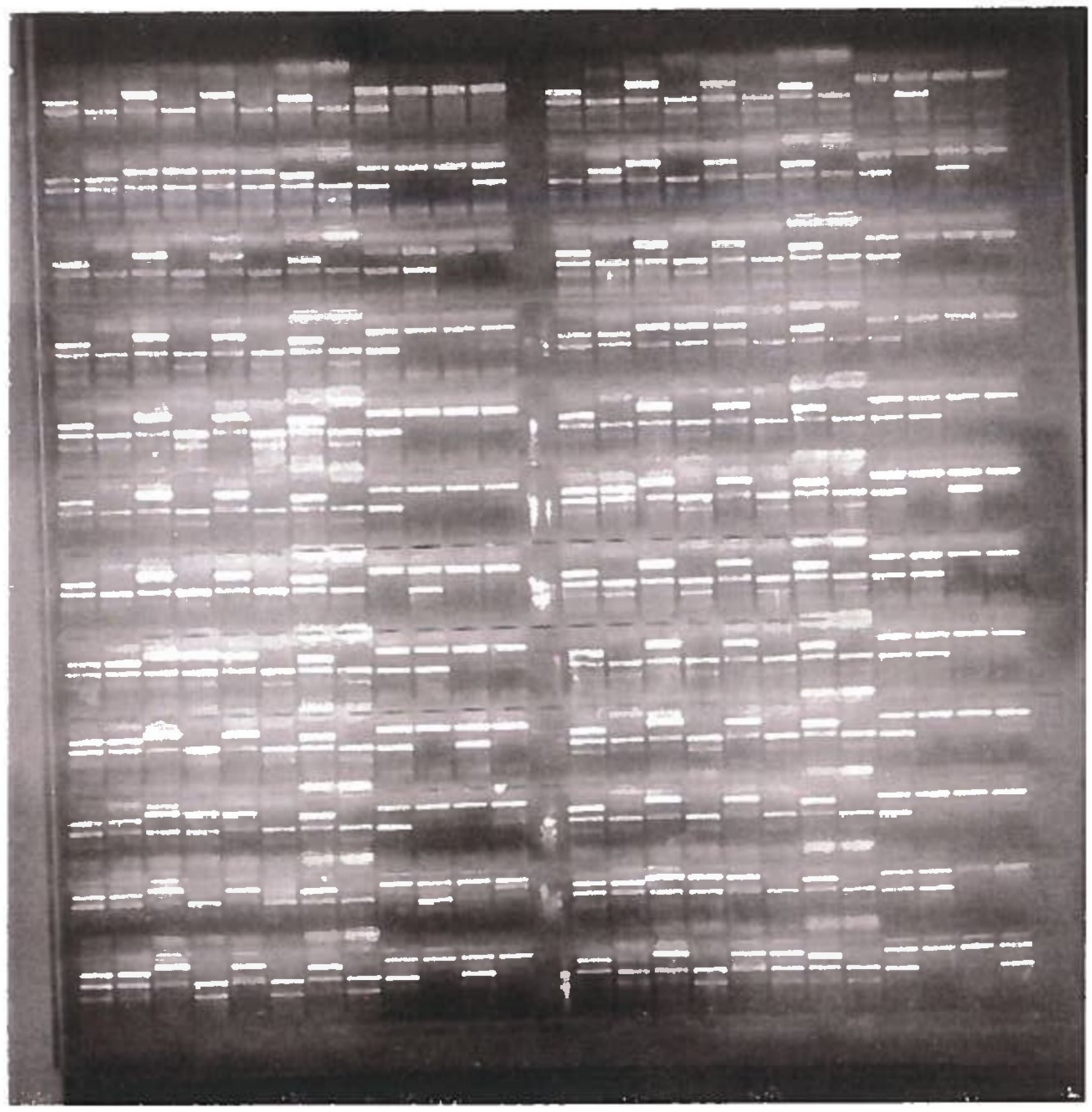


Fig.5.1 Gel electrophoresis of PCR products

5.4 Discussion

Associations between certain HLA types and an increased risk of skin cancer after transplantation have been reported. The associations have been explained by the difference in the ability to present cancer or viral antigens due to different conformations of the antigen-presenting groove. Our finding of an association between HLA B27 and the risk of BCC confirms previous

MHC class I region. If HPVs contribute to the pathogenesis of SCC and BCC in transplant recipients, as it is suggested by the high frequency of HPV DNA detection in these cancers then viral antigens would be presented by MHC class I molecules. Certain HLA alleles would be more efficient at presenting antigen due to specific aminoacids in critical positions in the antigen-presenting region or due to spatial conformation. We have been unable to show any association with any of the MHC class II molecules previously described in non-immunosuppressed patients. Following transplantation there is a strong increase in the risk of viral-associated diseases. It is possible that there is a stronger pathogenic contribution of HPV in renal transplant recipients compared with non-immunosuppressed patients.

Another interpretation of the HLA association with the risk of skin cancer suggests that the HLA alleles are a marker for the effect of other genes in the region. One prime candidate is TNF-α, which is the major inflammatory cytokine modulating immune responses following UV exposure. Previously TNF_{-308G} had been linked to an increased risk of cancer. However testing for 12 of the TNF alleles, TNF_{-1032C}, TNF_{-1032T}, TNF_{-865A}, TNF_{-865C}, TNF_{-859A}, TNF_{-859C}, TNF_{-380A}, TNF_{-380G}, TNF_{+488A}, TNF_{+488G}, TNF_{-238A}, TNF_{-238G}, TNF_{-308A}, TNF_{-308G} failed to show any association (Appendix A32 *TNF-α polymorphisms and skin cancer in RTRs)*. It is possible that indeed as it was suggested before, the association with the HLA alleles is true and is linked to the ability to present antigen. It is also possible that these polymorphic forms of the gene have no functional impact on the level of TNF-α production, or their effect is counteracted by the effect of another allele or indeed other alleles that we have not tested have an effect. I refer in particular to TNF_{-376A} which has been shown to influence the level of TNF expression (Knight, 1999 #362). It is without doubt that the parameters of the immune response are genetically determined and fine-tuning of this mechanism result from interactions between factors participating in the response cascade. It is only a matter of time to find out which genes are important in regulating these factors.

matter of time to find out which genes are important in regulating these factors.

Table 5.3 TNF-α polymorphisms and skin cancer in renal transplant recipients

TNF allele	Cases (%)	Controls (%)	P value
-1032T	110 (50.2)	109 (49.8)	0.376
-1032C	56 (57.1)	42 (42.9)	0.143
-865C	115 (51.1)	110 (48.9)	1.000
-865A	40 (56.3)	31 (43.7)	0.318
-859C	116 (50.7)	113 (49.3)	0.498
-859A	12 (44.4)	15 (55.6)	0.542
-380G	118 (51.1)	113 (48.9)	NA
-380A	2 (66.7)	1 (33.3)	1.000
+488G	104 (51.2)	99 (48.8)	1.000
+488A	2 (100)	0	0.498
-238G	101 (49.5)	103 (50.5)	0.222
-238A	0	0	NA
-308G	73 (48.7)	77 (51.3)	0.337
-308A	4 (50.0)	4 (50.0)	1.000

Chapter 6

Langerhans Cells, a Quantitative and Morphological Study in Renal Transplant Recipients

6.1 Introduction

Langerhans cells (LCs) are resident intraepidermal dendritic cells with a characteristic ultrastructural appearance in the skin and other stratified squamous epithelia. In human epidermis they occupy a basal and suprabasal position, and are present in a density of between 460-1000/mm^3. Langerhans cells only constitute 2% of all epidermal cells but through their long dendritic processes are able to provide a network capable of surveilling events within the whole epidermis. They express on their surfaces a number of antigens among which are CD1, CD4, MHC class II. Langerhans cells are the major antigen-presenting cells in the skin and it has been suggested that they may be able to initiate immune responses against tumour antigens (see section 4.1.1 *The skin immune system*). Ultraviolet radiation depletes transiently CD1+ DR+ LCs and impairs the ability of LCs to stimulate proliferative T cell responses in vitro (Baadsgaard *et al.*, 1989). *In vitro*, cyclosporine A was shown to inhibit antigen processing and accessory functions of LCs (Dupuy *et al.*, 1991; Furue & Katz, 1988). However *in vivo* studies in renal transplant recipients yielded conflicting results (see Chapter 2 *Skin cancer in renal transplant recipients: Pathogenesis).*

To evaluate the contribution of LCs in the pathogenesis of skin cancers we investigated the number and morphology of LCs in sun-exposed and non sun-exposed skin of renal transplant recipients with and without skin cancers, matched for age, gender, duration and type of immunosuppression and age- and gender-matched normal controls.

6.2 PATIENTS AND METHODS

Patients

The Central Oxford Research Ethics Committee (COREC 96.184) approved the study. Renal transplant recipients with and without skin cancer were identified from the skin cancer database. They were matched for gender, age at transplantation, type and duration of immunosuppression. Normal controls were age and gender matched volunteers with no history of skin cancer or other malignancy, immunosuppressive drug treatment or radiotherapy. All subjects recruited received information leaflets on skin biopsies and suction blisters (Appendix A.13 *Information leaflet on skin samples)* and prior to entry in the study signed a consent form (Appendix A.14 *Form of consent)*.

Skin biopsies

Two 4mm punch biopsies were taken simultaneously from a sun-exposed (dorsum of forearm) and non sun-exposed (inner arm) site, after local anaesthesia with 1% lignocaine. The specimens were snap frozen in liquid nitrogen and stored at -70° C. The donor sites was dressed with Kaltostat and Mepore. No complications have been reported following the procedure. Six-micrometer sections were cut and laid onto 10-well slides, which were stored at -70° C until staining. The slides were stained with a peroxidase antiperoxidase method according to the following ***protocol***:

1) Warm up slides at room temperature for 10 minutes
2) Fix slides in acetone for 10 minutes; air dry
3) One 10-minute wash in phosphate buffered saline (PBS)

4) Prepare primary antibodies

- CD1a mouse-antihuman (M0721, IgG2a; DAKO, Denmark) dilution 1:50,
- CD86 mouse anti-human (M7183, IgG1; DAKO, Denmark) dilution 1:25,
- CD25 (courtesy of Professor K.Gatter) dilution 1:1,
- MHC class II (courtesy of Professor K.Gatter) dilution 1:1,
- positive control anti GB3 (courtesy of Professor I.Leigh) dilution 1:100
- negative control (IgG 2a, X0943; DAKO, Denmark) dilution 1:50.

5) Incubate with 100µl/well of primary antibody for 45 minutes at room temperature in humidified tray

6) Prepare secondary antibody

- goat anti-mouse (P0447; DAKO, Denmark) dilution 1:100

7) Incubate with 100µl/well of secondary antibody for 45minutes at room temperature in humidified tray.

8) Wash x 3 for 10 minutes in PBS

9) Incubate with AEC substrate kit for peroxidase (SK 4200; Vector Laboratories Inc.) for 12 minutes

10) Wash for 10 minutes in distilled water

11) Counterstain for 2 minutes with Meyer's haematoxyllin (MHS-1; Sigma Diagnostics, USA)

12) Mount with Glycerol gelatin (GG1; Sigma Diagnostics, USA)

The number of LCs in vertical sections was counted per 10 basal layer cells in 3-5 fields.

Epidermal sheets

Two 6mm suction blisters were raised on a sun-exposed (dorsum of forearm) and non sun-exposed (inner arm) site. An old theatre suction machine (Fig.6.1) was adapted for this purpose by connection to a plastic tube and a bell-shaped glass bowl with 6mm orifices in the base. This was applied to the skin and secured with gauze (Fig.6.2). The pressure was increased progressively up to a

level that was comfortable for the patient for 30-90 minutes. The only side effects that occurred were slight itching when the epidermis began to separate from the dermis and mild bruising in a few patients with very thin skin. The blister was then de-roofed, the epidermis was snap frozen in liquid nitrogen and the site was dressed with Duoderm. The specimens were stored at -70°C until processing.

After thawing, epidermal sheets were later laid onto slides and double stained for CD1a and MHC class II by an indirect immunofluorescence technique according to the following ***protocol***:

1) Fix in acetone for 10 minutes at room temperature
2) Wash x 2 for 10 minutes in PBS
3) Prepare primary antibodies mixture

   CD1a mouse-antihuman (IgG2a, M0721; DAKO, Denmark) dilution 1:200 and

   MHC class II (IgG1, courtesy of Professor K.Gatter) dilution 1:1
4) Incubate with primary antibodies 100µl/well in a 96 well-plate, for 45' at room temperature
5) Wash x 3 in PBS
6) Prepare secondary antibodies mixture

   IgG1-FITC goat anti-mouse (1080-03, Southern Biotechnology Associates, Inc., USA) dilution 1:25 and

   IGG2a-TRITC goat anti-mouse (1070-02, Southern Biotechnology Associates, Inc., USA) dilution 1:50
7) 5 x 10 minute washes in PBS
8) Mount with Cityfluor (AF1, Agar Scientific)

The slides were then kept in the dark at 4°C until viewed with an Olympus fluorescence microscope. Epidermal sheets were first viewed under low power (x100) and any irregularities in the distribution of cells was noted. In each case positively stained cells were evenly distributed and therefore cells were counted in 5 random fields at a magnification of 400x using a graticule in the

eyepiece that measured an area of 0.25mm x 0.25mm (Graticules Ltd, Kent, UK). Results were presented as the mean number of CD1a positive cells/mm^2. Counting was done by a single investigator (CB).

Statistical analysis

Statistical analysis was done using the SPSS 10.0 software. Data was not normally distributes and therefore non-parametric tests (Wilcoxon signed rank test for paired data and Wilcoxon rank sum test for unpaired data) were used for analysis with the level of significance set at 0.05.

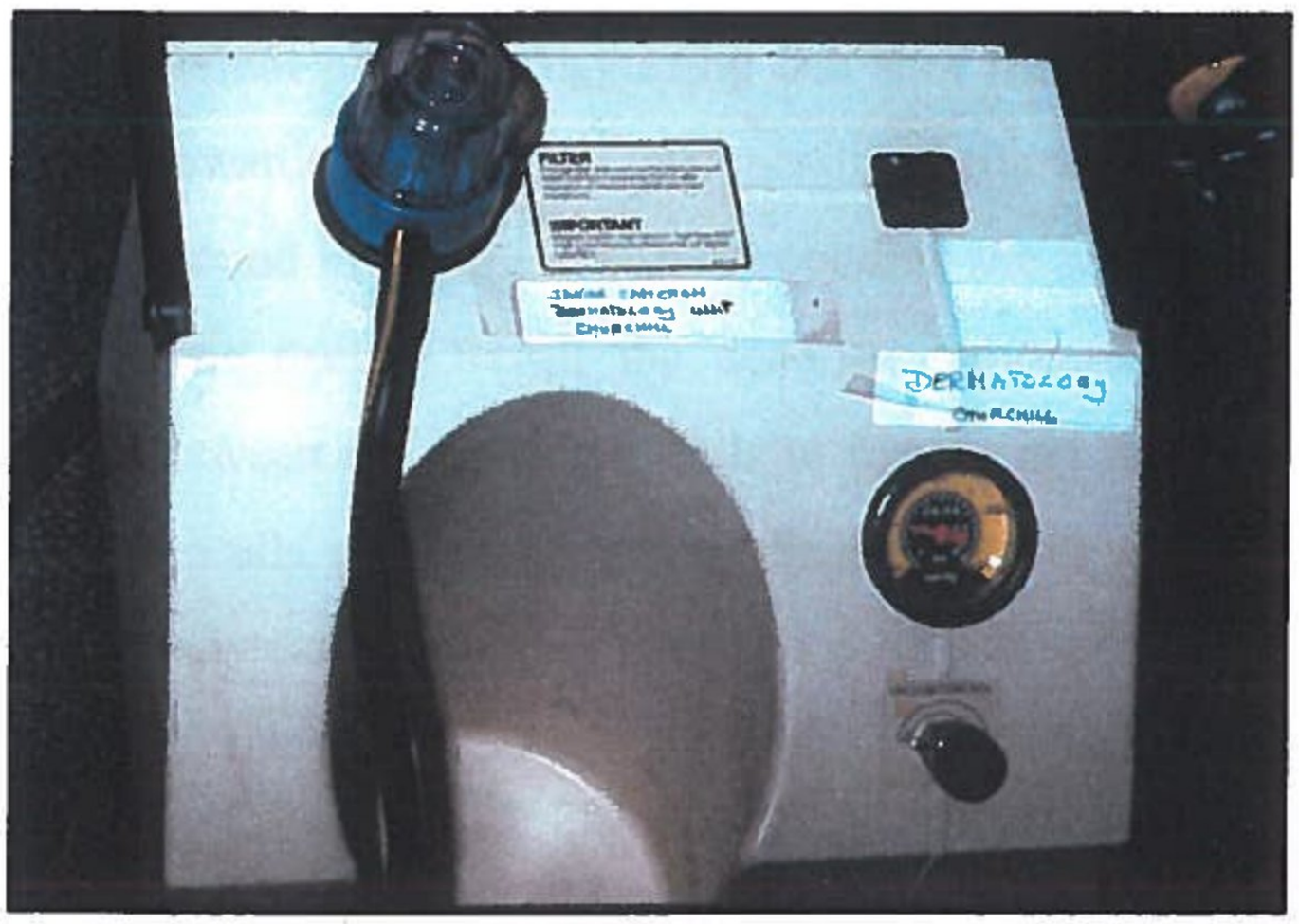


Fig.6.1 Machine used for raising suction blisters

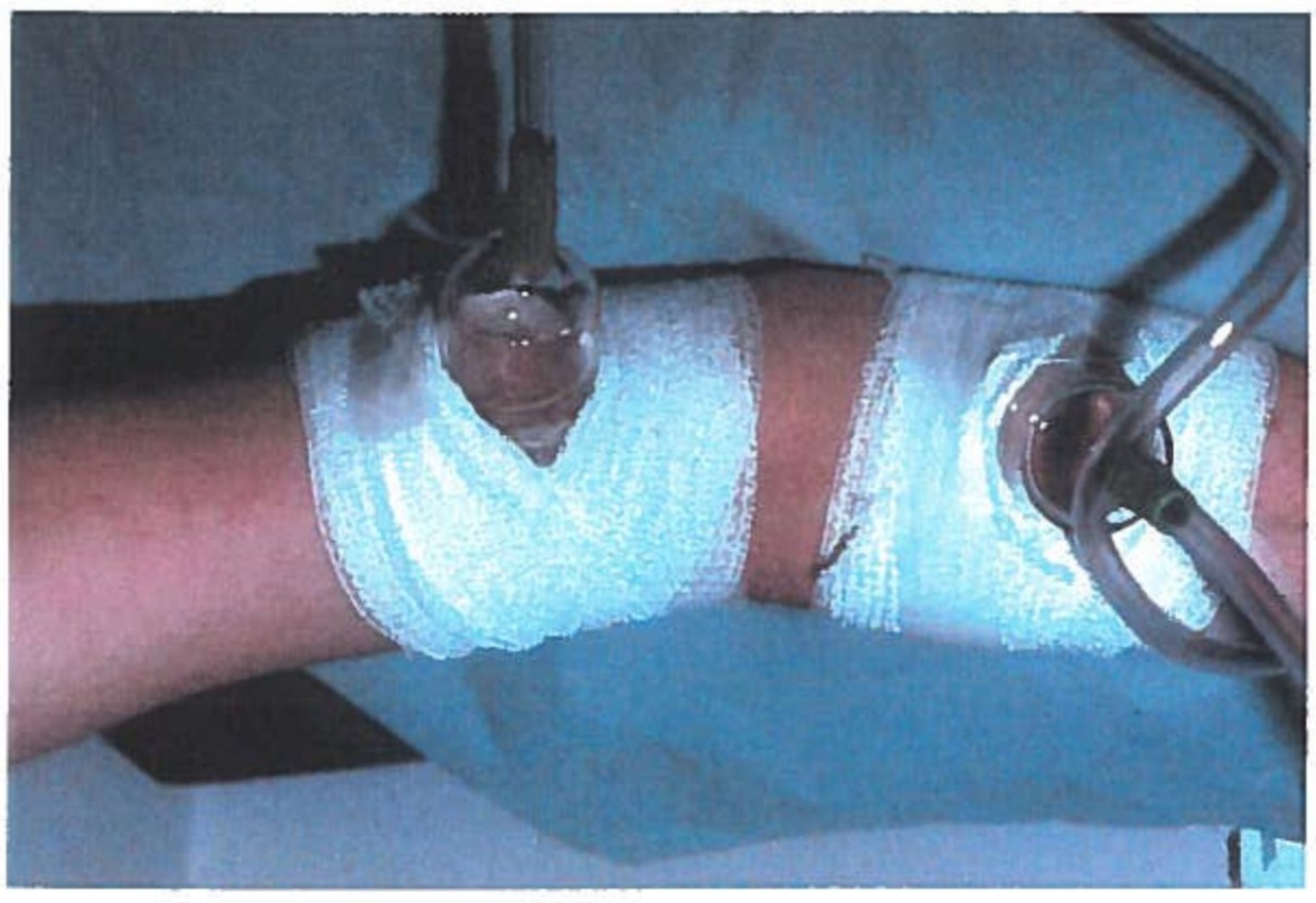


Fig.6.2 Suction bowls applied to skin

6.3 RESULTS

The results of the staining of vertical sections are presented in Table 6.1. None of the sections stained positive for CD86, CD25 or CD56 and these results were not listed. The number of CD1a positive cells is expressed as mean number of CD1a positive cells per 10 basal layer cells.

In all specimens CD1a positive cells were found in suprabasal or higher epidermal positions. On occasions CD1a positive cells were noticed at the epidermo-dermal junction suggesting that they may have migrated from the epidermis towards the dermis. CD1a positive cells were also noted around the hair follicles and in the epidermis surrounding the sebaceous glands or rarely in the dermis itself. Overall the dendritic processes of the CD1a+ cells extended between the epidermal cells to form a network but in a number of patients the cells were smaller with short dendrites. Although cells bodies were relatively easy to identify and with good counter-staining it was possible to count the number of CD1a+ cells per 10 basal layer cells, we considered this technique useful primarily for visualising the position of LCs in relation to the dermo-epidermal elements and less as an objective quantification method.

All patients had MHC class II positive cells and no particular pattern of distribution was noticed in relation to any of the groups. A number of patients stained positive for CD3 and/or CD4 and/or CD8. In all cases the T cell infiltrate was found in the dermis. Overall there were more patients who stained positive for T cell antigens and had more numerous cells in the transplant control group (group 3).

Table 6.1 Results of staining of vertical skin sections

Group	Site	CD1a	MHCII	CD3	CD4	CD8
1	FA	3.37	1+ (4) 2+ (6) 3+ (6)	2/16	1/16	1/16
	IA	3.46	1+ (5) 2+ (7) 3+ (4)	3/16	3/16	2/16
2.	FA	2.92	1+ (3) 2+ (4) 3+ (8)	3/15	2/15	1/15
	IA	3.23	1+ (2) 2+ (5) 3+ (8)	4/15	4/15	1/15
3.	FA	3.28	1+ (3) 2+ (7) 3+ (8)	3/18	3/18	2/18
	IA	3.51	1+ (0) 2+ (2) 3+ (15)	8/17	5/17	6/17
4.	FA	2.73	1+ (4) 2+ (3) 3+ (6)	0/13	0/13	0/13
	IA	3.15	1+ (3) 2+ (4) 3+ (6)	0/13	0/13	0/13
5.	FA	2.83	1+ (3) 2+ (6) 3+ (5)	1/14	1/14	0/14
	IA	3.39	1+ (4) 2+ (7) 3+ (3)	1/14	1/14	1/14

Gr.1-RTRs with SCCs; Gr.2-RTRs without SCCs; Gr.3-controls for Gr.1 and 2; Gr.4-non-immunosuppressed patients with SCCs; Gr.5-controls for Gr.4.

Staining of horizontal sheets provided a better mean of quantifying the number of CD1a positive cells and assessing the morphology. When the epidermal sheet was laid flat onto the slide we didn't encounter any major problems with

the depth of focus. After thawing, the epidermal sheets were flattened onto the slide under the microscope using microsurgical instruments. If the sheet was too large we divided it in two or three fragments, which were easier to manipulate. All CD1a+ cells were also MHC class II positive (Fig.6.3) and the results of CD1a cell counting are presented in Table 6.2 and Fig.6.4 and 6.5.

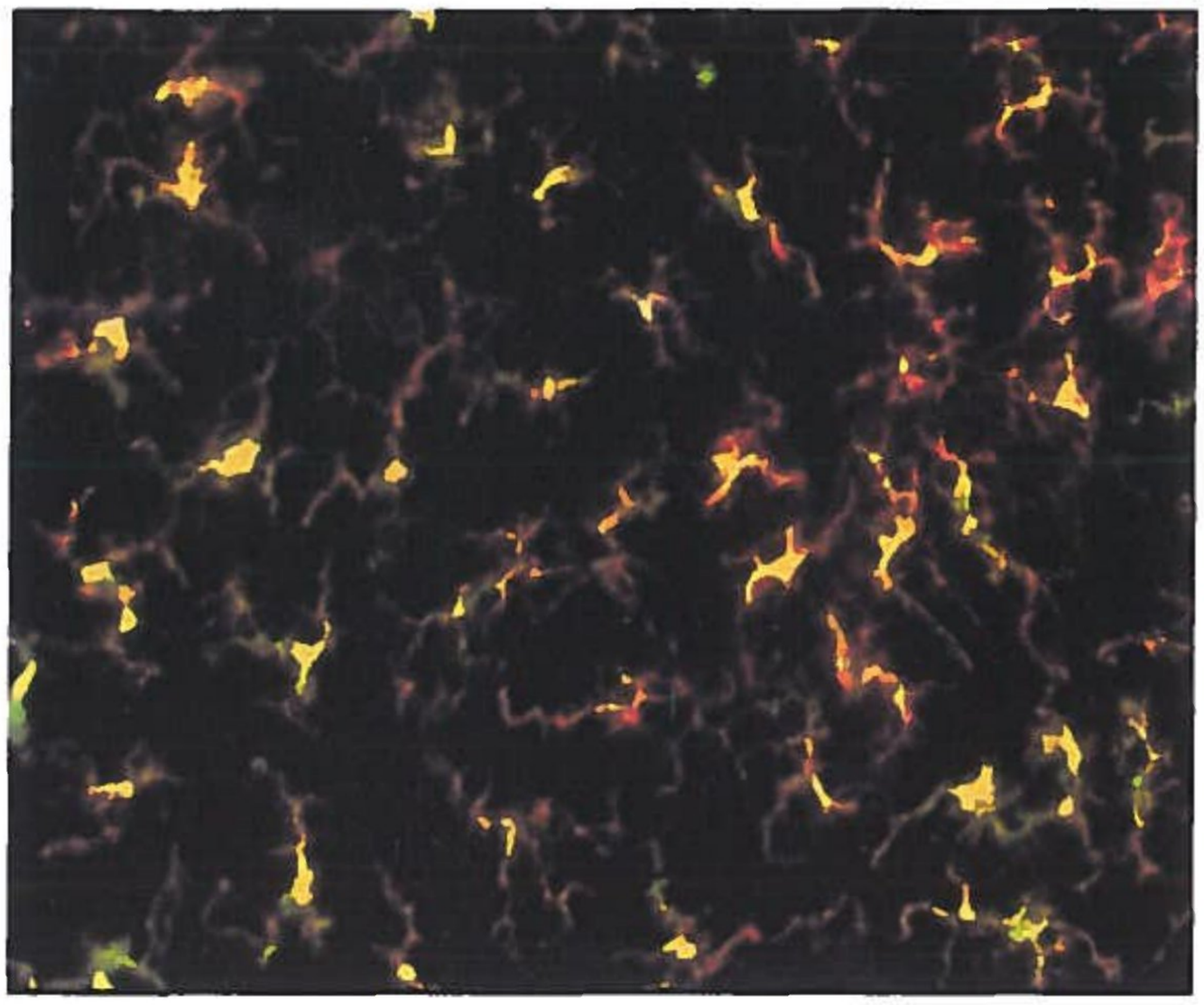


Fig.6.3 CD1a-MHC class II double staining of epidermal sheet

Table 6.2 Results of CD1a counting in epidermal sheets

Group	Site	Mean	Range	95% CI	Median
1	FA	462.9	333-600	389.5-536.2	450.0
	IA	451.7	283-750	360.1-542.6	442.0
2	FA	374.0	250-567	283.5-464.5	333.0
	IA	398.0	300-500	340.6-455.4	416.0
3	FA	558.5	417-667	456.7-660.3	558.5
	IA	545.3	367-667	447.6-642.9	583.0
4	FA	537.5	417-617	398.4-676.6	558.0
	IA	555.0	467-633	500.5-609.5	567.0
5	FA	536.6	433-650	427.7-645.5	550.0
	IA	462.0	333-550	390.3-533.7	467.0

Gr.1-RTRs with SCCs; Gr.2-RTRs without SCCs; Gr.3-controls for Gr.1 &2; Gr.4-non-immunosuppressed patients with SCC; Gr.5-controls for Gr.4.

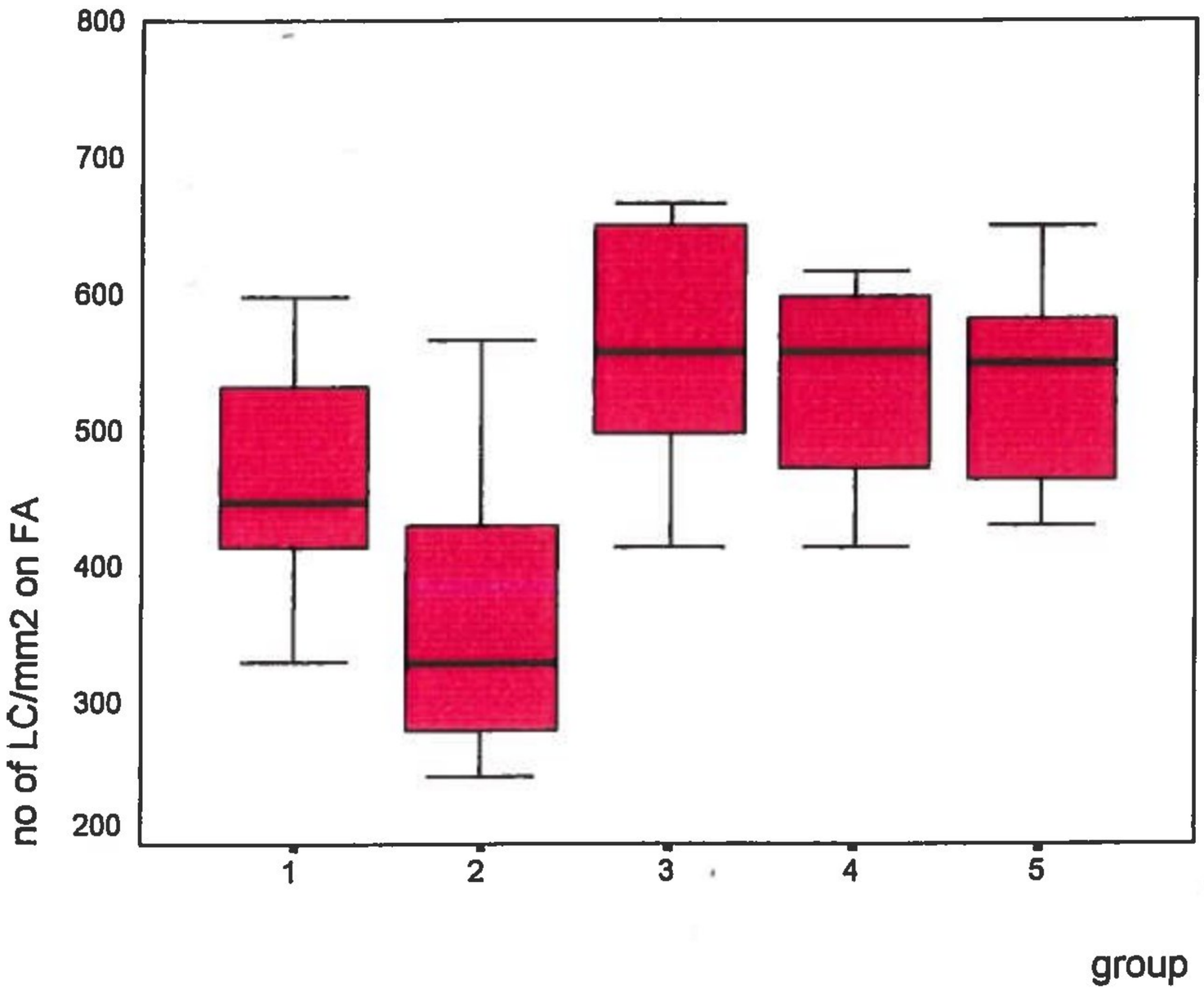


Fig. 6.4 CD1a counts in epidermal sheets on the forearm

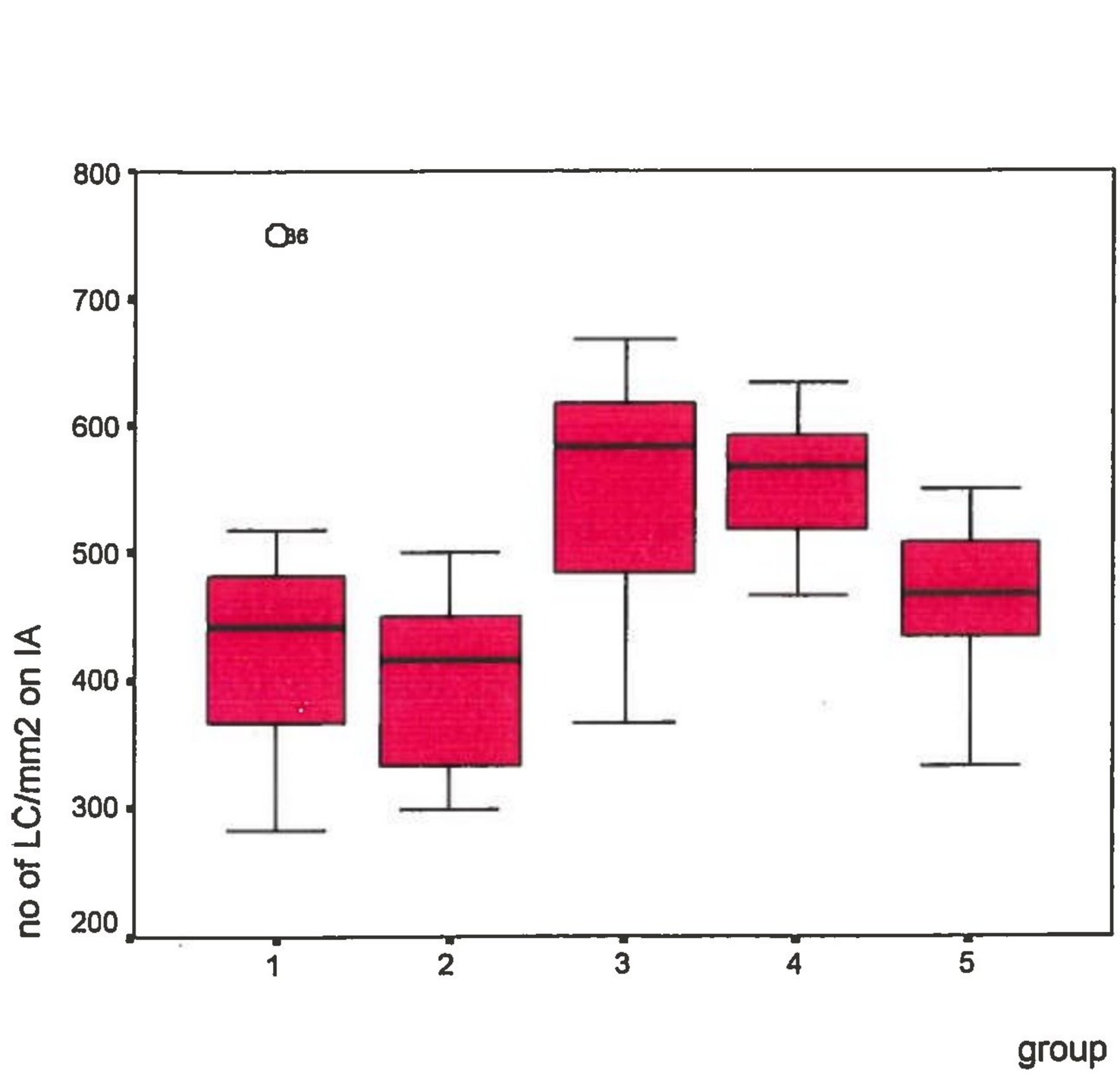


Fig.6.5 CD1a cell counts in epidermal sheets on the inner arm

Transplant patients with (group 1) and without SCCs (group 2) had a lower CD1a count both on the forearm and inner arm compared with the control group (group 3). The difference between groups 2 and 3 reached statistical significance both for the forearm ($p=0.02$) and inner arm ($p=0.02$). Non-immunosuppressed patients with SCC (group 4) had similar CD1 counts with the control group (group 5) on the forearm but had a lower count on the inner arm ($p=0.03$).

There were no significant CD1 count differences between the forearm and inner arm within the individual groups.

CD1a+ cells of transplant patients appeared more often rounded with short and smooth-rounded dendrites. Most CD1a+ cells in normal patients had long and fine dendrites interconnecting with the neighbouring cells' dendrites.

6.4 Discussion

Langerhans cells are the major antigen-presenting cell in the skin and it has been suggested that they may be involved in tumour antigen presentation. Both ultraviolet exposure and long term exposure to immunosuppression have been reported to lower the number and affect the function of Langerhans cells in the skin, potentially facilitating tumour development. Sontheimer *et al.* (Sontheimer *et al.*, 1984) observed a reduction in ATPase-positive cells in transplant recipients, which was greater in sun-exposed skin suggesting that immunosuppressive therapy potentiates the effect of ultraviolet exposure on the density of Langerhans cells. Servitje *et al.*(Servitje *et al.*, 1991) examined non sun-exposed skin of transplant patients and reported a reduction in the number of LCs compared to normal controls. In contrast Kelly *et al.*(Kelly *et al.*, 1987) found no difference in LCs in non sun-exposed skin between RTRs and normal controls. Data from these studies is difficult to interpret due to nonhomogeneous designs.

We have therefore investigated the number of CD1a-MHC class II positive cells in a matched case-control study. Renal transplant patients in the study were matched for age and duration and type of immunosuppression allowing to study only the effect of immunosuppression per se on the risk of skin cancer after transplantation.

Vertical sections of skin were stained for CD1a to study the position of Langerhans cells in relation to the basal cells and other dermal and epidermal cells and not for quantification purposes. However the counts of CD1a cells between groups were similar to the counts of CD1a cells in epidermal sheets. Cell morphology and dendricity were difficult to assess accurately on sections. Both immunosuppressed and non-immunosuppressed patients had dermal infiltrates of MHC class II positive cells with no particular distribution between groups. No patient stained positive for CD86 (present on resting monocytes and dendritic cells and activated T, natural killer and B cells), CD25 (IL2-R, present on activated lymphocytes) and CD56 (present on natural killer cells).

Staining of horizontal epidermal sheets allowed us the most accurate quantification of LCs and assessment of cell and dendritic morphology. All CD1a+ cells stained positive for the MHC class II antigen. Cell counts were within the ranges previously reported in the literature. CD1a cell counts in the transplant patients with SCCs were non-significantly lower compared to the normal controls both on the forearm and inner arm. However, CD1a cells count in transplant patients without SCCs were significantly lower compared with the normal controls and non-significantly lower when compared with the transplant patients with SCCs. The absence of a significant difference in LCs number between sun-exposed and non sun-exposed skin in our study could be due to the fact that most of the skin specimens were harvested between October- June when the levels of sun exposure are reduced. Similarly Kelly *et al.* who harvested their specimens during the winter months found no significant difference between RTRs and normal controls.

There was no difference in CD1a counts between non-immunosuppressed patients with SCC and matched controls on the forearm. On the inner arm the count was higher in the non-immunosuppressed patients with SCC than their controls. It is difficult to find a valid biological explanation for this difference, which was not mirrored by the count on the forearm and it is possible to be due to experimental factors.

It is tempting to speculate in RTRs with SCCs higher numbers of LCs are mobilised in the epidermis compared with the RTRs without skin cancers, but these cells are functionally deficient and therefore unable to fulfil their antigen-presenting function. It may not be an accidental finding that the highest CD1a cell count occurred in a RTR with numerous previous skin cancers. Although the morphology of LCs was noticed to be altered in transplant patients compared with non-immunosuppressed patients an objective assessment of the surface area of cells and dendricity did not form the object of this study.

In conclusion the number of Langerhans cells cannot be used to predict the risk of skin cancers in renal transplant patients. It is possible that further functional studies may be able to demonstrate an impairment in LC function in RTR with skin cancer.

Chapter 7

Conclusions

Since the early seventies it was noticed that renal transplant recipients develop an increased number of skin cancers, most of which are squamous cell carcinomas. While high incidences have been reported from areas with high levels of sun exposure, reports from transplant centres in Europe indicated that the number of patients affected who live in a temperate climate is much smaller. Our personal experience in a dedicated dermatology clinic at the Transplant Centre in Oxford prompted us to study the epidemiology of skin cancers which have occurred in the Oxford transplant population over the last 21 years, to investigate in vivo immune responses, Langerhans cells numbers and genetic factors like HLA and TNFα polymorphisms in patients with and without skin cancers. Almost all patients transplanted in Oxford have been followed up in the Renal Transplant Centre and there is accurate information regarding their skin cancers, treatment, HLA types and multiple DNA samples available. Furthermore skin problems have been closely followed up in a dedicated clinic and skin cancers have been diagnosed histologically by a pathologist with a special interest in dermatopathology. This population therefore represented an ideal setting for this type of study.

In summary the findings and contributions of this thesis are:

1. **Chapter 3** ***Skin cancers in renal transplant recipients: A single-centre, 21-year retrospective analysis***
   - During data collection a unique and extensive database on skin cancers has been created which will represent an essential source of information for future studies.

- Our population is the largest to be studied in Britain and one of the largest from Europe (to our knowledge) and we recorded all types of skin cancers (including Bowen's disease).
- The epidemiological study looked in detail at elements not previously reported (time to presentation in relation to the tumour type and gender, anatomical distribution for individual tumour types, distribution of skin cancers in relation to the fistula side) to identify any clues related to biological behaviour.
- We showed that the rate of skin cancers following transplantation is higher than previously reported from Europe. The cumulative risk of skin cancer in comparable to figures previously published from Australia (61% at 22 years post transplant).
- We have calculated incidence rates for individual skin cancer types and relative risks for malignant melanoma and squamous cell carcinoma.
- We investigated 49 variables and four risk factors were identified in a multivariate analysis: increased age at first transplantation, male gender, total time-exposure to immunosuppression and higher creatinine levels at 1 year post transplant. Based on this model it is possible to estimate the risk of skin cancer for individual patients and select those who will need close follow up.

2. **Chapter 4** *The role of immunological factors in the aetiology of squamous cell carcinoma in renal transplant recipients*
   - We have compared antigen specific and nonspecific immune responses in a case control study in transplant patients with and without skin cancer, non-immunosuppressed patients with skin cancer and normal controls. No case control study with stringent matching had been done previously. Transplant patients were matched for age, gender, duration and type of immunosuppression. Patients were not matched for the cumulative doses of immunosuppressive drugs, as this was the variable we wanted to investigate in relation to the qualitative difference in immune responses. However cumulative doses were calculated for each

patient at the end of the study. Every effort was made to maximise the sample size. Sample size was limited by the number of cases with and without skin cancer still alive and able to spare the time for the study.

- Our findings suggest that renal transplant patients with skin cancer have lower delayed type antigen-specific immune responses and require stronger antigenic stimuli to achieve a response compared to renal transplant patients without skin cancer. This would indicate that there are qualitative differences in the degree of immunosuppression between patients with and without skin cancer.
- Non specific inflammatory responses to dithranol were higher in transplant patients with skin cancer than transplant patients without skin cancer. This could be due to defective antioxidant mechanisms in patients with skin cancer.
- Nonspecific inflammatory responses to sodium lauryl sulphate were comparable between the groups of transplant patients although slightly lower compared to the normal controls.
- The finding of lower antigen specific immune responses in patients with skin cancer is a strong argument to investigating further whether it is possible to tailor individually the amount of immunosuppression (eg. based on metaboliser profiles, trough levels) that would prevent rejection and cause the minimum skin side effects.

3. **Chapter 5** *HLA types and TNFα polymorphisms and skin cancer risk in renal transplant recipients*
   - We have confirmed the association between HLA B27 and the risk of basal cell carcinoma previously reported from both Europe and Australia.
   - We have found a novel association between HLA A17 and the risk of squamous cell carcinoma.
   - No association was found between the TNFα alleles studied and the risk of skin cancer. It is possible that: a) the association with HLA alleles is primary and is linked to the ability to present antigen; b) other TNFα

alleles are linked to the risk of skin cancer; c) the candidate gene TNFα is not implicated at all and the HLA association is secondary to a third gene (not HLA, not TNF) in the MHC region.

4. **Chapter 6** *Langerhans cells, a quantitative and morphological study in transplant patients*

- Our hypothesis of a lower number of Langerhans cells in transplant patients with skin cancers compared to transplant patients without skin cancer was not confirmed.
- Morphologically Langerhans cells in transplant patients had fewer and shorter dendrites compared to the non-immunosuppressed patients but there was no qualitative difference between transplant patients with and without skin cancers.
- The number of Langerhans cells cannot be used to predict the risk of skin cancer in renal transplant recipients. Further functional studies are required to see whether these Langerhans cells are efficient antigen presenting cells.

Appendix A

A.1 Demographic variables

Name	
Hospital number	
Gender	male/female
Date of birth	dd/mm/yy
Place of birth	
Date of transplantation[1]	dd/mm/yy
Date of failure[1]	dd/mm/yy
Cause of end-stage renal failure	
Date of death	dd/mm/yy
Cause of death	
Number of transplants	
Dialysis before the first transplant	Y/N
Haemodialysis	Y/N
Peritoneal dialysis	Y/N
Date start dialysis	dd/mm/yy
Ever smoked	Y/N
Smoked before first transplant	Y/N
Smoked after first transplant	Y/N
Recipient blood group	O/A/B/AB
Recipient Rh	positive/negative
Type of immunosuppression	
Total units of blood transfusion (Tissue Typing database)	

[1]date of transplantation and failure of each transplant

A.2 TRANSPLANT-RELATED VARIABLES

Units of perioperative blood transfusion	
Recipient CMV status	negative/positive
Mismatches for A locus	0/1/2
Mismatches for B locus	0/1/2
Mismatches for Bw locus	0/1
Mismatches for C locus	0/1/2
Mismatches for DR locus	0/1/2
Mismatches for DQ locus	0/1/2
Pretransplant antibodies	Y/N
High sensitisation	Y/N
Post-transplant dialysis	Y/N
Warm ischaemia time	
Cold ischaemia time	
Number of rejection episodes	
Antilymphocyte globulin	Y/N
Methylprednislone	grams
OKT3	Y/N
Relation	cadaveric/living-related
Days to failure	
Donor gender	male/female
Donor age	
Donor blood group	O/A/B/AB
Donor Rh	positive/negative
Donor CMV status	negative/positive
Graft source	local/ UKTTS
Creatinine at 3 month post-transplant	
Creatinine at 1 year post-transplant	
Creatinine at 2 years post-transplant	
Creatinine at 4 years post-transplant	
Creatinine at 5 years post-transplant	
Creatinine at 10 years post-transplant	

A.3 SKIN CANCER DATA

Histopathology report number	
Date when specimen received	dd/mm/yy
Histological diagnosis	
Anatomical site	
Completeness of excision	completely/incompletely excised

A.4 SKIN CANCERS: HISTOLOGICAL TYPES

Skin cancer type	**Number of tumours (%)**
Squamous cell carcinoma	540 (50.7)
Bowen's disease	246 (23.1)
Basal cell carcinoma	169 (15.9)
Keratoacanthoma	70 (6.6)
Malignant melanoma	4 (0.4)
Others	36 (3.4)
Total	1065

A.5 SKIN CANCER: ANATOMIC DISTRIBUTION

Anatomical site	**Number of tumours (%)**
Head	384 (36.0)
Neck	54 (5.1)
Chest	66 (6.2)
Abdomen	11 (1.0)
Back	50 (4.7)
Upper limb except hand	126 (11.8)
Hand	238 (22.3)
Lower limb except foot	87 (8.2)
Foot	12 (1.1)
Unspecified	37 (3.5)
Total	1065

A.6 SKIN CANCER: ANATOMIC DISTRIBUTION OF INDIVIDUAL SKIN CANCER TYPES

Skin cancer type	Anatomic site (number of tumours and %)										
	H[1]	N[2]	C[3]	A[4]	UL[5]	H[6]	LL[7]	F[8]	B[9]	NK[10]	Total
Squamous cell carcinoma	230(42.6)	25(4.6)	18(3.3)	4(0.7)	50(9.3)	148(27.4)	32(5.9)	7(1.3)	10(1.9)	16(2.9)	540
Bowen's disease	53(21.5)	12(4.9)	24(9.8)	3(1.2)	36(14.6)	38(15.4)	52(21.1)	3(1.2)	18(7.3)	7(2.8)	246
Basal cell carcinoma	85(50.3)	10(5.9)	19(11.2)	3(1.8)	12(7.1)	9(5.3)	4(2.4)	1(0.6)	18(10.7)	8(4.7)	169
Keratoacanthoma	8(11.4)	5(7.1)	4(5.7)	1(1.4)	22(31.4)	26(37.1)	1(1.4)	0	2(2.9)	1(1.4)	70
Malignant Melanoma	0	0	0	0	2	0	1	0	0	1	4
Others	8	2	1	0	4	11	2	1	1	6	36

[1]Head; [2]Neck; [3]Chest; [4]Abdomen; [5]Upper limb except hand; [6]Hand; [7]Lower limb except foot; [8]Foot; [9]Back; [10]unspecified.

A.7 HISTOLOGICAL TYPE OF FIRST SKIN CANCER TO PRESENT

Skin cancer type	Number of patients(%)
Squamous cell carcinoma	68(36.4)
Basal cell carcinoma	41(21.9)
Bowen's disease	38(20.3)
Keratoacanthoma	15(8.0)
Squamous cell carcinoma and Bowen's disease	9(4.8)
Squamous cell and basal cell carcinoma	3(1.6)
Malignant melanoma	2(1.1)
Others	11(5.9)
Total	187

A.8 COX'S UNIVARIATE ANALYSIS OF PATIENT-RELATED VARIABLES

Variable	Odds ratio (95% CI)	p value
Age at first transplantation	1.06 (1.05-1.07)	<0.0001
Age group at first transplantation		
35-44 years old	2.51 (1.57-4.02)	0.001
45-54 years old	4.59 (2.92-7.20)	<0.0001
>55 years old	9.47 (5.80-15.45)	<0.0001
Gender (females)	0.68 (0.49-0.93)	0.016
Dialysis before 1st transplantation (Yes)	0.97 (0.61-1.54)	0.896
Dialysis type before 1st transplantation		
Haemodialysis only	0.98 (0.59-1.56)	0.894
Peritoneal dialysis only	0.99 (0.56-1.75)	0.966
Both haemo- and peritoneal dialysis	0.98 (0.58-1.66)	0.947
Length of dialysis before 1st transplantation	1.05 (0.94-1.18)	0.362
Smoking ever (Yes)	1.13 (0.84-1.53)	0.423
Smoking before 1st transplantation (Yes)	1.15 (0.85-1.56)	0.367

Smoking after 1st transplantation (Yes)	0.76 (0.48-1.21)	0,252
Primary renal disease		
Glomerular (reference)		
Interstitial	1.00	
Hereditary	0.66 (0.44-1.00)	0.053
Vascular	1.21 (0.81-1.81)	0.348
Diabetes	1.06 (0.62-1.82)	0.836
Primary renal disease	0.14 (0.12-1.03)	0,053
Others		
Number of renal grafts	1.02 (0.37-2.79)	0,973
1 graft (reference)		
2 grafts	1.00	
>3 grafts	0.54 (0.36-0.82)	0.004
Recipient ABO blood group	0.49 (0.22-1.12)	0,090
A (reference)		
AB	1.00	
B	0.92 (0.40-2.10)	0.843
O	0.70 (0.39-1.26)	0.233
Recipient Rhesus antigen status (positive)	1.33 (0.84-1.54)	0.419
Immunosuppression "era" (Cya)[1]	1.21 (0.79-1.85)	0.370
	1.77 (1.21-2.59)	0,003

[1]for patients with one transplantation only

A.9 COX'S UNIVARIATE ANALYSIS OF TRANSPLANT-RELATED VARIABLES

Variable	Odds ratio (95%CI)	P value
Blood transfusion (> 3 units)	0.87 (0.64-1.19)	0.390
Perioperative transfusion (Yes)	0.88 (0.66-1.19)	0.422
Recipient CMV status (positive)	1.17 (0.85-1.62)	0.329
Mismatches for A locus		
0 mismatches (reference)	1.00	
1 mismatch	1.28 (0.88-1.85)	0.196
2 mismatches	1.30 (0.85-1.98)	0.221
Mismatches for B locus		
0 mismatches (reference)	1.00	
1 mismatch	1.31 (0.88-1.93)	0.183
2 mismatches	1.04 (0.68-1.59)	0.846
Mismatches for Bw locus (1 mismatch)	1.06 (0.76-1.48)	0.732
Mismatches for C locus		
0 mismatches (reference)	1.00	
1 mismatch	1.04 (0.74-1.47)	0.803
2 mismatches	0.98 (0.59-1.62)	0.932
Mismatches for DR locus		
0 mismatches (reference)	1.00	
1 mismatch	0.95 (0.69-1.32)	0.776
2 mismatches	0.91 (0.59-1.41)	0.674
Mismatches for DQ locus		
0 mismatches (reference)	1.00	
1 mismatch	1.07 (0.70-1.64)	0.739
2 mismatches	0.52 (0.13-2.14)	0.366
Pre-transplant antibodies (Yes)	1.04 (0.77-1.40)	0.814
High sensitisation (Yes)	1.14 (0.77-1.69)	0.495
Warm ischaemia time (≠0)	0.52 (0.37-0.71)	<0.0001
Cold ischaemia time (>21.2 hours)	1.56 (1.15-2.09)	0.004
Antilymphocyte globulin (Yes)	0.94 (0.56-1.57)	0.799

Methylprednisolone (>1.6 g)	0.72 (0.53-0.97)	0.031
Post-transplant dialysis (Yes)	0.83 (0.59-1.16)	0.267
Number of acute rejection episodes		
0 episodes (reference)	1.00	
1 episode	0.94 (0.63-1.39)	0.764
2 episodes	0.84 (0.55-1.28)	0.415
≥3 episodes	0.66 (0.42-1.04)	0.074
Relation (living related)	0.45 (0.21-0.97)	0.041
OKT 3 (Yes)	1.17 (0.37-3.69)	0.784
Cycosporine A (Yes)	1.43 (1.01-2.02)	0.046
Donor age	1.02 (1.01-1.03)	0.0001
Donor gender (females)	1.24 (0.92 –1.67)	0.159
Donor ABO blood group		
A (reference)	1.00	
AB	0.61 (0.15-2.49)	0.491
B	0.44 (0.20-0.96)	0.039
O	1.03 (0.76-1.39)	0.831
Donor Rhesus antigen status (positive)	0.84 (0.57-1.25)	0.393
Donor CMV status (positive)	1.33 (0.97-1.83)	0.076
Source of the graft		
Local (reference)	1.00	
UKTS	0.97 (0.69-1.34)	0.840
Creatinine at 3 months (>150μmol/l)	1.24 (0.81-1.89)	0.317
Creatinine at 1 year (>150 μmol/l)	1.65 (1.20-2.27)	0.002
Creatinine at 2 years (>150μmol/l)	1.39 (0.99-1.96)	0.061
Creatinine at 3 years (>150μmol/l)	1.58 (1.09-2.26)	0.013
Creatinine at 4 years (>150μmol/l)	1.33 (0.91-1.95)	0.144
Creatinine at 5 years (>150μmol/l)	1.31 (0.89-1.91)	0.172
Creatinine at 10 years (>150μmol/l)	1.39 (0.89-2.14)	0.141

A.10 COMPARATIVE SKIN CANCER RATES AND TIME TO PRESENTATION IN RELATION TO THE IMMUNOSUPPRESSIVE REGIMEN

Variable	Cyclosporine "era"	Azathioprine "era"
Number of skin cancers	379	477
Number of squamous cell carcinomas (%)	165 (43.5)	255 (53.5)
Number of basal cell carcinomas (%)	85 (22.4)	60 (12.6)
Number of Bowen's disease (%)	112 (29.6)	97 (20.3)
Total time on immunosuppression	3273.13 years	2480.1 years
Rate of skin ca	116/1000 py[1]	192/1000 py
Rate of squamous cell carcinoma	50/1000 py	103/1000 py
Rate of basal cell carcinoma	26/1000 py	24/1000 py
Rate of Bowen's disease	34/1000 pyr	39/1000 py
SCC/BCC	1.94/1	4.25/1
Mean time to presentation (SD)	5.2 (3.2) years	10 (3.4) years

[1]py=person year

A.11 LETTER OF INVITATION (1)

Oxford Radcliffe HOSPITALS

OXFORD DERMATOLOGY
TREATMENT · CARE · INNOVATION

THE CHURCHILL

DERMATOLOGY DEPARTMENT
Old Road
Headington
Oxford
OX3 7LJ

Enquiries: 01865-228232
Appointments: 01865-228206
Clinical FAX: 01865-228240
Ward 01865-228250

Consultants:
Dr. R. P. R. Dawber
01865-228265

Dr. F. Wojnarowska
01865-228266

Dr. S. M. Powell
01865-228205

Dr. N. P. J. Walker
01865-228224/37

Dr. S. Burge
01865 228258

Senior Nurse
Miss J. Merryweather
01865-228250

Clinical Measurement
Dr. G. W. Cherry
01865-228269/38

Laser Service
Mrs Cheryl Day
01865 228223

Medical Records
01865-228204

Business Directorate:

SDU Director
Dr. S. M. Powell

Research Director
Dr. G. W. Cherry

SDU Manager
Mrs. Cheryl Day

Clinical Centre Manager
Mrs. Linda Kenedy
01865-225243

Admin FAX
01865-228260

...../...../.....

Dear ,

The Oxford Transplant Centre and the Dermatology Department at the Churchill Hospital are undertaking a study of Skin Immune Responses in relation to Skin Cancer. We are commencing a major study in an attempt to identify some of the factors contributing to the development of skin cancers. Our study groups include patients with and without skin cancers.

There are two parts to the study. The first part involves skin allergy testing and the second part involves taking small samples of skin from one of your arms. You will find enclosed two information sheets giving you further details of what this involves.

We will be contacting you in the near future to see if you would be willing to participate in either one or both parts of the study. In the meantime if you would like to discuss any aspect of the study or require further information please call Dr. Cristina Bordea (research fellow) or Janice Cameron (research nurse) on Tel: 01865 228230, who will be pleased to answer any questions you might have.

There is no obligation to participate and if you do not wish to take part in the study your treatment will not be affected in any way.

Yours sincerely,

Professor Sir Peter J Morris
Director Transplant Centre

Dr. Fenella Wojnarowska
Consultant Dermatologist

The Oxford Radcliffe Hospital
A National Health Service Trust

A.12 INFORMATION LEAFLET ON SKIN TESTS

FW/CB

PATIENT INFORMATION (Allergy testing)

Dear Patient

Study of Skin Immune Responses in relation to Skin Cancer

We are asking if you could help us with a study that involves some simple allergy testing on the skin of your arm and back. This is to see whether reactions in patients without skin cancer are different from those with skin cancer. This would involve a number of different tests: all of them have been used for years in medical practice.

1. *Patch Tests*: This involves sticking one strip of paper with five itchy substances onto the skin of your back. Each substance is placed onto a small (10mm) disc. This is used routinely in skin clinic. These patches would remain on your back for 2 days. We would see them at that time and again in a further 2 days. The only reaction that you may have will be itching locally, which will rapidly disappear.

One substance would be applied onto one arm/ back for 48 hours with sticky tape. Four weeks later the same substance would be applied to both arm/ back for 6 hours. We would look at the test site at 2 and 4 days. Itching would be the only reaction that you might have.

2. *Multitest*: We would test your response to 7 common infections in the environment by using the multitest, the allergens are pricked into the skin and the response seen after 2 days.

3. *Irritant reading*: Some 3 substances are mildly irritating to the skin, we would test how much your skin responds by applying them exactly as we do the patch test, using 3 strips of paper.

4. *Skin samples* (Some patients only): You would be asked to consider having a biopsy of the skin (see separate information sheet). This is to study the cells in the skin responsible for the immunity.

During the time that the patch tests are being performed (5 days) you cannot wash your upper back/ arms, but this would be the only restriction to your normal day to day life.

You are free to decide not to take part in this study and you can leave it at any time. This would not influence the medical care that you receive.

If you have any problems do not hesitate to contact us on: (01865) 228230.

PLEASE KEEP THIS INFORMATION SHEET IN CASE YOU NEED TO PRESENT IT TO A DOCTOR DURING THE TESTS.

Yours sincerely,

Dr.F Wojnarowska
Consultant Dermatologist

Prof. Sir P J Morris
Director Transplant Centre

Central Oxford Research Ethics Committee Project No: 96.184

A.13 INFORMATION LEAFLET ON SKIN SAMPLES

FW/CB

PATIENT INORMATION (Skin samples)

Dear Patient,

Study of Skin Immune Responses in relation to Skin Cancer

As part of a study of skin immune responses in relation to skin cancer we are asking if you would help us by having small samples of skin taken from one of your arms. Two different procedures would be used (both have been used for many years in clinical practice):

1. *Suction blisters:* We would ask you to allow us to make 4 small blisters on the skin of your forearm. Mild suction is applied to the skin, which lifts the very top layer of the skin off. This takes between 30 and 90 minutes in most people. You may sit and/ or lie down whilst this is done. There is usually brief itching just before the blisters appear. We would then remove the top of each blister for study. Small raw areas, about 6mm in diameter would be left, which would usually heal within a week, but there may be some discoloration, which would slowly fade.

This is approximately 6mm: ●

2. *Skin biopsy*: You would be asked to consider having 2 small biopsies of the skin. This is to study the cells in the skin responsible for immunity. Two small pieces of skin 4mm in diameter would be removed using local anaesthetic. Stitches are not usually required and complications such as infection are very rare. There would be 2 small scars.

This is approximately 4mm: ●

You would need a covering plaster or dressing over the blister or biopsy site for a few days.

You are free to decide not to take part in this study and you could leave it at any time. This would not influence the medical care that you receive.

If you have any problems please do not hesitate to contact us on: (01865) 228230.

PLEASE KEE THIS INFORMATION SHEET IN CASE YOU NEED TO PRESENT IT TO A DOCTOR DURING THE TESTS.

Yours sincerely,

Dr. F.Wojnarowska
Consultant Dermatologist

Prof Sir P. J. Morris
Director Transplant Centre

Central Oxford Research Ethics Committee Project No: 96.184

A.14 FORM OF CONSENT

Oxford Radcliffe HOSPITAL

Appointments 01865 225355/6
Transplant Ward 01865 226122/11
Fax 01865 225616

Our Ref:

OXFORD TRANSPLANT CENTRE
THE CHURCHILL
Headington
Oxford
OX3 7LJ

Director
Professor Sir Peter J Morris
01865 225761

Consultants
Mr DWR Gray
01865 226092

Mr JA Rouke
01865 225357

Miss A Robertson
01865 226092

Mr D Cranston (Urology)
01865 225941

Immunologist
Dr Ken Welsh
01865 226162

Senior Nurse Practitioner & SDU Manager
Miss PM Franklin
01865 225761

Nephrologists
Professor P Ratcliffe
01865 225761

Dr P Altmann
01865 225357

Dr P Mason
01865 225802

Dermatologist
Dr F Wojnarowska
01865 225761

FORM OF CONSENT

Title of Project: Studies of Skin Cancer and the Prevalence of Human Papilloma Virus in Cutaneous Lesions

COREC No: 96.184

(The patient should complete the rest of this sheet)

Have you read the Invitation Letter/ Information Sheet ? Yes/ No

Have you had an opportunity to ask questions and discuss this study ? Yes/ No

Have you received satisfactory answers to your questions ? Yes/ No

Have you received enough information about the study ? Yes/ No

Who you have spoken to ? Dr/ Mr/ Mrs.....

Do you understand that you are free to leave the study:
- at any time
- without having to give a reason for leaving
- and without affecting your medical care ? Yes/ No

Signed:..... Date: .....

Name (in block letters):.....

The Oxford Radcliffe Hospital
A National Health Service Trust

A.15 LETTER OF INVITATION (2)

Oxford Radcliffe HOSPITAL

OXFORD DERMATOLOGY
TREATMENT · CARE · INNOVATION

THE CHURCHILL

DERMATOLOGY DEPARTMENT
Old Road
Headington
Oxford
OX3 7LJ

Dear

We are undertaking a study of skin immune responses in an attempt to identify some of the factors contributing to the development of skin cancers. Our study groups include patients with and without a history of skin cancers.

The study involves some simple skin testing by the application of small patches to the skin. We would also ask you if you would be willing to have two small skin samples taken from your arm under local anaesthetic.

During the skin tests we would ask you to either lie down on a couch or lean forward while we take readings at the test sites with a sensor that lightly rests on your skin. During this time you could listen to music. Volunteers who have already participated in the study have found it very relaxing.

We would like to ask you if you would be interested in helping us with the study. If you would like further information please could you fill in the enclosed form and return it in the stamped addressed envelope. If there is any aspect of the study you would like to discuss please call Janice Cameron on 01865 228230 and leave a message with your name, telephone number and a suitable time to call you back.

There is no obligation to participate and if you do not wish to take part in the study any treatment that you may require in the future will not be affected in any way.

Dr. Fenella Wojnarowska
Consultant Dermatologist

Dr.Cristina Bordea
Clinical Research Fellow

Enquiries: 01865-228232
Appointments: 01865-228206
Clinical FAX: 01865-228240
Ward 01865-228250

Consultants:
Dr. R. P. R. Dawber
01865-228265

Dr. F. Wojnarowska
01865-228266

Dr. S. M. Powell
01865-228205

Dr. N. P. J. Walker
01865-228224/37

Dr. S. Burge
01865 228258

Senior Nurse
Miss J. Merryweather
01865-228250

Clinical Measurement
Dr. G. W. Cherry
01865-228269/38

Laser Service
Mrs Cheryl Day
01865 228223

Medical Records
01865-228204

Business Directorate:

SDU Director
Dr. S. M. Powell

Research Director
Dr. G. W. Cherry

SDU Manager
Mrs. P. Tennant
01865-228262

General Manager
Mrs. Jane Imbush
01865-225243

Admin FAX
01865-228260

A.16 PATIENT REPLY SLIP

If you would like further information about the study, please fill in this form and return in the stamped addressed envelope provided.

Or alternatively telephone Janice Cameron on: 01865 228230

Thank you

I would like further information about the skin cancer study

Name..... Mr/Mrs/Ms

Date of birth ...../...../.....

Address .....

.....

Post code ....

Tel: ....

Time available on above number .....

Skin cancer study

There are two parts to the study:

- one involves some simple allergy testing on the skin
- the other involves taking two small samples of your skin (from your inner arm and your forearm), for us to study.

(*The skin samples are less than the size of the base of a small pencil)*

The study requires **4 visits** as follows:

1st visit one patch test is applied and skin samples are taken. (visit time approximately one and a half hours)

Approximately 4- 6 weeks after the 1st visit **2nd, 3rd & 4th visit** take place on the Monday, Wednesday and Friday of the same week when patch tests are undertaken.

You should allow approximately 1 1/2 hours for each visit.

Visit times are arranged to suit the participants and can be ***any time between 8am and 7pm***. However we do need you to attend at the same time on each of the 3 days in the week of the tests (or within an hour of that time).

If you would like further information about the study, please fill in the attached form and leave at the reception desk.

Alternatively please contact Janice Cameron or Christina Bordea Tel: 01865 228230 (answer phone out of hours)

Thank you for your interest.

I would like further information about the skin cancer study

Name.....Mr/Mrs/Miss

Date of birth ...../...../.....

Address .....

.....

Post code .....

Tel: .....

Time available on above number .....

A.18 DNCB: MEAN ERYTHEMA READING

Site	Time	Group 1 Mean (med*, SD)	Group 2 Mean (med, SD)	Group 3 Mean (med, SD)	Group 4 Mean (med, SD)	Group 5 Mean (med, SD)
Control	Baseline	78.8 (77.6, 25.1)	85.3 (80.6, 22.9)	73.3 (76.8, 25.3)	89.7 (89.2, 25.9)	65.6 (64.8, 17.6)
	48h	76.8 (77.2, 23.3)	85.7 (78.6, 26.2)	74.9 (71.8, 28.3)	90.7 (86.0, 26.2)	65.4 (65.4, 17.8)
	96h	80.2 (82.2, 30.3)	85.4 (82.2, 28.7)	71.2 (72.4, 23.6)	93.6 (85.2, 26.8)	64.9 (63.8, 18.7)
2.2g	baseline	NA	NA	87.4 (87.4, 17.5)	89.6 (89.0, 18.4)	74.7 (71.2, 20.6)
	48h	NA	NA	105.6 (88.2, 42.2)	100.7 (93.0, 25.9)	78.3 (81.2, 17.4)
	96h	NA	NA	120.1 (91.0, 60.5)	97.4 (85.4, 35.9)	96.5 (80.4, 45.1)
3.125g	baseline	NA	NA	82.2 (81.0, 17.6)	82.5 (83.4, 21.3)	68.0 (73.8, 18.5)
	48h	NA	NA	102.9 (90.6, 41.9)	91.7 (87.6, 21.2)	70.2 (71.0, 23.0)
	96h	NA	NA	119.9 (105.8, 55.1)	95.1 (98.2, 23.4)	96.0 (71.0, 50.3)
4.325g	baseline	NA	NA	84.9 (72.4, 36.6)	83.7 (83.8, 17.8)	68.2 (71.6, 18.4)
	48h	NA	NA	123.5 (113.0, 56.0)	108.9 (103.6, 44.2)	132.8 (117.6, 69.4)
	96h	NA	NA	163.6 (185.2, 73.3)	131.3 (111.4, 71.5)	170.3 (181.0, 90.0)
6.25g	baseline	86.8 (90.8, 26.0)	91.7 (91.2, 19.1)	75.4 (63.2, 30.9)	75.2 (75.8, 19.8)	61.4 (67.8, 17.5)
	48h	88.7 (93.8, 25.0)	103.9 (97.4, 31.8)	138.1 (122.8, 65.5)	130.9 (131.8, 60.5)	142.7 (124.2, 77.2)
	96h	86.5 (78.0, 31.2)	95.2 (87.0, 24.0)	160.3 (157.4, 65.5)	161.6 (179.4, 86.0)	171.9 (176.8, 82.7)
8.75g	baseline	75.8 (84.0, 24.2)	83.9 (83.2, 22.7)	NA	NA	NA
	48h	78.7 (82.6, 23.8)	111.8 (109.0, 39.7)	NA	NA	NA
	96h	80.5 (82.6, 31.2)	102.7 (87.0, 38.0)	NA	NA	NA
12.5g	baseline	84.8 (82.0, 19.4)	87.9 (84.4, 19.2)	NA	NA	NA
	48h	93.1 (98.0, 23.2)	112.9 (113.2, 35.1)	NA	NA	NA
	96h	90.8 (87.9, 33.2)	114.2 (111.4, 33.2)	NA	NA	NA
17.7g	baseline	79.4 (75.2, 24.6)	81.1 (77.4, 29.0)	NA	NA	NA
	48h	109.6 (91.6, 44.7)	128.8 (132.8, 45.7)	NA	NA	NA
	96h	116.6 (90.1, 62.5)	123.2 (130.6, 45.8)	NA	NA	NA

*median

A.19 DNCB: RESULTS OF THE LINEAR MIXED EFFECTS MODEL ANALYSIS FOR ΔE

Site	Time	2 vs. 1*	3 vs. 1*	3 vs. 2*	5 vs. 4*	4 vs. 1*
C	48 h	0.62	0.41	0.75	0.76	0.51
	96 h	0.63	0.36	0.71	0.35	0.90
2.2μg	48 h	NA	NA	NA	0.21	NA
	96 h	NA	NA	NA	0.25	NA
3.125μg	48 h	NA	NA	NA	0.24	NA
	96 h	NA	NA	NA	0.22	NA
4.325μg	48 h	NA	NA	NA	0.03	NA
	96 h	NA	NA	NA	0.03	NA
6.25μg	48 h	0.17	<0.001	<0.001	0.20	<0.001
	96 h	0.85	<0.001	<0.001	0.35	<0.001
8.75μg	48 h	0.02	NA	NA	NA	NA
	96 h	0.41	NA	NA	NA	NA
12.5μg	48 h	0.05	NA	NA	NA	NA
	96 h	0.05	NA	NA	NA	NA
17.7μg	48 h	0.17	NA	NA	NA	NA
	96 h	0.95	NA	NA	NA	NA

*1,2,3,4 and 5 refer to patient groups 1,2,3,4,5. The figures in the table represent p-values of the paired comparisons between the groups. The analysis tested the null hypothesis that there is no difference between the groups.

A.20 DNCB: LINEAR MIXED EFFECTS MODEL ANALYSIS OF ΔSFT

Site	Time	2 vs. 1	3 vs. 1	3 vs. 2	5 vs. 4	4 vs. 1
C	48 h	0.4	1.0	0.2	0.8	0.6
	96 h	0.3	0.3	0.6	0.6	0.8
2.2µg	48 h	NA	NA	NA	1.0	NA
	96 h	NA	NA	NA	0.7	NA
3.125µg	48 h	NA	NA	NA	0.8	NA
	96 h	NA	NA	NA	0.1	NA
4.325µg	48 h	NA	NA	NA	0.1	NA
	96 h	NA	NA	NA	0.1	NA
6.25µg	48 h	0.3	<0.001	<0.001	0.4	<0.001
	96 h	0.9	<0.001	<0.001	0.2	<0.001
8.75µg	48 h	0.1	NA	NA	NA	NA
	96 h	0.4	NA	NA	NA	NA
12.5µg	48 g	0.4	NA	NA	NA	NA
	96 g	0.5	NA	NA	NA	NA
17.7µg	48 h	0.5	NA	NA	NA	NA
	96 h	0.9	NA	NA	NA	NA

A.21 DITHRANOL: MEAN ERYTHEMA READINGS

Site	Time	Group 1 Mean (med*, SD)	Group 2 Mean (med, SD)	Group 3 Mean (med, SD)	Group 4 Mean (med, SD)	Group 5 Mean (med, SD)
Control	baseline	101.1 (98.6, 26.0)	104.4 (98.8, 32.9)	113.8 (110.0, 41.8)	107.2 (109.4, 30.6)	86.3 (85.6, 16.5)
	48h	101.1 (113.6, 30.1)	100.6 (100.2, 25.8)	106.8 (103.6, 39.0)	100.1 (100.0, 29.2)	82.7 (81.2, 23.3)
	96h	96.4 (96.4, 25.2)	91.3 (92.3, 23.0)	107.4 (100.4, 37.6)	98.9 (101.1, 23.8)	83.4 (83.0, 19.0)
WSP	baseline	97.5 (105.2, 24.6)	107.1 (97.6, 39.0)	111.4 (99.8, 35.1)	108.0 (102.6, 35.1)	86.4 (86.8, 13.1)
	48h	87.0 (96.6, 24.6)	95.8 (92.8, 28.7)	104.3 (104.4, 34.3)	95.0 (81.8, 38.2)	80.1 (77.4, 28.3)
	96h	98.4 (90.2, 23.5)	93.4 (92.4, 32.0)	112.0 (102.2, 33.0)	102.7 (98.4, 24.8)	89.9 (90.4, 27.0)
0.0025%	baseline	106.0 (114.8, 33.4)	113.6 (111.0, 37.7)	122.7 (111.8, 42.6)	120.8 (124.4, 38.8)	101.3 (109.0, 25.2)
	48h	123.4 (125.0, 33.8)	109.5 (105.0, 35.2)	122.3 (118.8, 38.1)	132.3 (131.6, 34.7)	107.5 (100.8, 28.5)
	96h	138.7 (135.7, 46.3)	116.9 (107.6, 33.7)	139.4 (146.6, 45.2)	137.1 (137.5, 28.7)	117.3 (120.8, 30.0)
0.005%	baseline	100.6 (97.8, 27.0)	106.6 (102.0, 42.6)	120.2 (117.0, 37.3)	113.7 (118.4, 36.5)	95.7 (92.0, 17.4)
	48h	114.6 (115.8, 29.9)	113.5 (112.0, 35.4)	127.9 (122.8, 38.8)	132.3 (135.6, 38.9)	110.8 (101.8, 36.0)
	96h	136.2 (137.8, 38.3)	119.5 (114.4, 36.1)	143.8 (139.6, 52.2)	154.1 (146.7, 36.6)	135.1 (128.0, 39.8)
0.01%	baseline	96.4 (98.8, 25.0)	111.1 (99.0, 40.1)	113.6 (115.8, 35.0)	110.4 (114.8, 29.8)	93.7 (97.0, 18.6)
	48h	129.6 (129.4, 38.1)	129.1 (127.6, 36.4)	138.9 (136.4, 41.4)	157.2 (159.2, 32.2)	134.4 (124.2, 36.4)
	96h	169.1 (163.3, 44.3)	149.5 (155.2, 35.8)	177.3 (179.2, 52.9)	193.0 (189.4, 41.2)	180.2 (185.8, 43.3)

*median

A.22 DITHRANOL: RESULTS OF MIXED EFFECTS MODEL FOR ΔE

Site	Time	2 vs. 1	3 vs. 2	3 vs. 1	4 vs. 5	4 vs. 1
Control	48 h	NS	NS	NS	NS	NS
	96 h	NS	NS	NS	NS	NS
WSP	48 h	NS	NS	NS	NS	NS
	96 h	0.01	NS	NS	NS	NS
0.0025%	48 h	0.03	NS	NS	NS	0.07
	96 h	0.001	NS	0.002	NS	0.002
0.005%	48 h	NS	NS	NS	NS	NS
	96 h	0.004	NS	NS	NS	NS
0.01%	48 h	NS	NS	NS	NS	NS
	96 h	0.01	NS	NS	NS	NS

A.23 DITHRANOL: MEAN TEWL READINGS

Site	Time	Group 1 Mean (median, SD)	Group 2 Mean (median, SD)	Group 3 Mean (median, SD)	Group 4 Mean (median, SD)	Group 5 Mean (median, SD)
Control	baseline	12.6 (6.7, 20.4)	8.2 (7.4, 4.6)	5.0 (4.1, 3.5)	5.4 (5.7, 2.5)	5.2 (4.9, 2.1)
	48h	12.3 (7.6, 19.6)	6.5 (6.5, 3.3)	4.0 (3.2, 2.8)	4.4 (3.6, 2.4)	4.7 (5.3, 1.7)
	96h	12.5 (7.1, 21.6)	6.7 (4.8, 4.6)	3.9 (3.9, 2.7)	4.2 (3.8, 2.8)	4.4 (4.5, 1.8)
WSP	baseline	12.7 (7.7, 19.6)	8.1 (7.7, 4.3)	4.8 (3.8, 3.2)	5.4 (5.4, 2.7)	5.4 (5.6, 2.2)
	48h	12.5 (7.8, 18.6)	7.1 (5.8, 4.5)	4.6 (3.7, 2.9)	5.1 (5.4, 2.9)	4.8 (4.3, 2.1)
	96h	12.4 (6.6, 21.3)	6.3 (5.0, 3.4)	3.8 (4.3, 2.3)	4.4 (3.5, 2.4)	4.5 (4.1, 2.1)
0.0025%	baseline	13.4 (9.4, 16.1)	8.1 (7.1, 4.7)	5.8 (5.2, 2.8)	6.3 (6.2, 2.3)	6.5 (7.0, 1.9)
	48h	14.2 (10.3, 15.6)	9.1 (7.9, 4.8)	6.5 (6.0, 3.1)	7.7 (7.4, 2.8)	7.2 (7.0, 2.2)
	96h	13.5 (8.5, 16.6)	7.5 (6.3, 3.6)	5.4 (5.7, 2.2)	6.5 (6.6, 2.5)	5.5 (5.8, 1.3)
0.005%	baseline	13.2 (8.5, 17.7)	8.1 (6.3, 7.1)	5.2 (3.9, 3.4)	6.3 (6.0, 2.4)	6.2 (5.9, 2.2)
	48h	12.4 (8.5, 16.8)	8.9 (7.3, 4.4)	5.3 (4.5, 3.4)	7.3 (7.0, 2.9)	6.8 (6.1, 2.7)
	96h	12.7 (7.7, 18.9)	7.4 (6.6, 3.3)	4.2 (3.5, 2.2)	5.7 (5.2, 2.8)	5.3 (4.8, 2.0)
0.01%	baseline	13.1 (8.0, 18.7)	7.8 (7.1, 5.0)	4.9 (4.6, 2.9)	5.9 (5.6, 2.9)	5.8 (5.2, 2.4)
	48h	13.3 (7.7, 18.1)	8.7 (8.3, 4.6)	5.5 (4.5, 3.9)	7.0 (6.3, 3.5)	6.0 (5.2, 2.2)
	96h	13.6 (7.7, 20.2)	6.9 (4.9, 3.7)	4.2 (3.6, 2.4)	5.3 (4.3, 3.2)	4.8 (4.3, 2.0)

A.24 DITHRANOL: MEAN CORNEOMETER READINGS

Site	Time	Group 1 Mean (median, SD)	Group 2 Mean (median, SD)	Group 3 Mean (median, SD)	Group 4 Mean (median, SD)	Group 5 Mean (median, SD)
C	baseline	85.3 (51.5, 96.5)	91.3 (74.5, 52.0)	87.9 (74.5, 52.0)	76.0 (70.0, 40.3)	85.8 (80.0, 37.4)
	48h	97.7 (85.0, 54.5)	122.4 (103.0, 62.3)	130.4 (116.0, 60.3)	116.9 (120.0, 55.8)	128.5 (123.0, 41.1)
	96h	75.7 (63.0, 46.2)	96.2 (80.0, 45.1)	110.1 (86.0, 62.0)	93.4 (86.0, 51.3)	111.2 (103.0, 32.6)
WSP	baseline	85.2 (60.0, 97.0)	87.4 (71.5, 51.7)	90.8 (81.5, 54.6)	77.2 (73.0, 35.1)	84.0 (80.0, 36.5)
	48h	134.4 (148.0, 49.2)	154.9 (148.0, 55.1)	189.9 (183.0, 88.8)	175.2 (176.0, 77.3)	185.1 (163.0, 59.2)
	96h	80.3 (83.0, 24.7)	97.4 (80.0, 38.7)	128.1 (103.0, 63.4)	104.9 (101.5, 48.7)	133.8 (126.0, 41.5)
0.0025%	baseline	83.6 (51.5, 83.8)	76.7 (73.0, 36.3)	94.1 (80.0, 54.2)	73.6 (66.0, 35.6)	80.0 (66.0, 36.5)
	48h	133.5 (123.0, 69.0)	152.1 (151.0, 59.9)	192.3 (196.0, 71.8)	168.2 (166.0, 77.1)	192.4 (186.0, 66.1)
	96h	73.9 (70.0, 31.4)	103.1 (96.0, 43.7)	119.7 (103.0, 65.6)	101.8 (91.5, 59.5)	126.2 (123.0, 40.0)
0.005%	baseline	86.1 (56.5, 98.4)	86.1 (76.5, 46.8)	90.8 (78.0, 57.5)	73.3 (76.0, 24.0)	86.0 (76.0, 42.8)
	48h	136.1 (131.5, 64.7)	173.4 (164.5, 75.7)	183.2 (176.0, 105.5)	166.6 (163.0, 73.2)	190.6 (180.0, 50.8)
	96h	78.3 (73.0, 30.0)	107.2 (103.0, 38.5)	128.9 (103.0, 72.8)	103.8 (104.5, 40.2)	139.2 (133.0, 47.0)
0.01%	baseline	85.7 (58.0, 95.7)	80.1 (73.0, 42.5)	84.7 (73.0, 51.9)	74.8 (76.0, 26.6)	87.8 (80.0, 42.2)
	48h	132.8 (119.5, 62.8)	167.1 (153.0, 67.8)	183.5 (173.0, 92.5)	183.8 (186.0, 60.4)	207.4 (193.0, 65.4)
	96h	85.9 (73.0, 35.6)	107.5 (96.0, 42.6)	132.4 (113.0, 65.2)	111.4 (126.5, 39.8)	145.7 (140.0, 43.8)

A.25 DITHRANOL: MIXED EFFECTS MODEL ANALYSIS OF ΔCM

Site	Time	2 vs. 1	3 vs. 2	3 vs. 1	4 vs. 5	4 vs. 1
Control	48h	NS	NS	NS	NS	NS
	96h	NS	NS	NS	NS	NS
WSP	48h	NS	NS	0.05	NS	NS
	96h	NS	NS	NS	0.06	NS
0.0025%	48h	0.08	NS	0.006	NS	0.02
	96h	NS	NS	NS	NS	NS
0.005%	48h	0.05	NS	NS	NS	NS
	96h	NS	NS	NS	0.05	NS
0.01%	48h	0.02	NS	0.004	NS	0.001
	96h	NS	0.04	0.08	0.08	NS

A.26 SLS: MEAN ERYTHEMA READINGS

Site	Time	Group 1 Mean (median, SD)	Group 2 Mean (median, SD)	Group 3 Mean (median, SD)	Group 4 Mean (median, SD)	Group 5 Mean (median, SD)
Control	baseline	108.4 (111.0, 33.7)	122.5 (125.0, 43.4)	126.9 (121.6, 31.8)	130.4 (127.6, 38.7)	126.8 (126.0, 25.9)
	48h	113.6 (105.8, 42.4)	121.8 (124.8, 40.6)	121.3 (116.8, 35.3)	124.6 (123.0, 32.7)	122.5 (116.2, 24.2)
	96h	105.8 (109.4, 35.5)	133.2 (119.8, 29.1)	123.6 (121.6, 39.2)	124.9 (127.4, 37.6)	122.3 (107.2 (35.8)
SW	baseline	111.5 (113.4, 37.1)	127.8 (120.6, 46.7)	135.5 (137.6, 32.8)	138.3 (139.2, 34.3)	139.6 (144.2, 29.8)
	48h	116.4 (110.8, 39.0)	133.2 (131.4, 38.6)	142.9 (154.8, 35.5)	148.0 (158.8, 34.3)	140.9 (142.6, 30.9)
	96h	119.0 (114.6, 42.4)	129.6 (122.8, 36.8)	146.1 (146.0, 36.7)	148.8 (157.0, 36.2)	140.2 (145.4, 28.5)
0.0625%	baseline	124.5 (119.8, 41.3)	117.6 (109.0, 37.3)	138.0 (144.4, 51.5)	129.7 (139.2, 34.6)	112.0 (110.4, 23.6)
	48h	125.8 (128.6, 32.7)	132.7 (135.6, 34.3)	149.8 (158.6, 51.3)	146.2 (156.8, 36.6)	127.3 (128.0, 26.6)
	96h	131.8 (125.0, 38.7)	130.5 (127.7, 32.6)	163.9 (155.0, 46.3)	150.6 (154.2, 43.2)	132.5 (133.8, 30.8)
0.125%	baseline	121.3 (114.2, 36.5)	114.9 (110.0, 39.1)	128.8 (126.2, 40.0)	127.2 (133.2, 36.4)	123.9 (120.6, 26.0)
	48h	125.6 (128.0, 31.1)	130.3 (135.0, 43.2)	146.9 (159.0, 43.6)	138.9 (142.0, 42.8)	133.2 (133.0, 36.7)
	96h	133.8 (133.0, 41.5)	136.1 (142.3, 34.5)	161.8 (163.2, 37.9)	164.5 (174.2, 33.9)	152.6 (150.2, 33.9)
0.25%	baseline	113.4 (107.0, 39.2)	130.9 (124.8, 38.4)	140.3 (135.4, 43.3)	151.3 (142.4, 40.8)	132.8 (132.4, 23.7)
	48h	129.3 (134.0, 45.7)	147.1 (146.4, 39.7)	159.7 (162.0, 50.0)	160.0 (167.8, 44.3)	148.1 (144.8, 42.8)
	96h	139.2 (141.6, 38.1)	150.6 (151.4, 36.4)	180.1 (186.6, 44.4)	178.4 (162.0, 53.8)	168.0 (174.6, 31.6)
0.5%	baseline	117.0 (109.6, 39.1)	119.3 (125.8, 37.8)	131.7 (126.8, 50.1)	129.8 (136.2, 28.8)	126.3 (119.6, 25.2)
	48h	142.9 (152.6, 42.0)	155.7 (161.8, 48.1)	175.4 (190.8, 50.1)	166.9 (167.8, 32.7)	166.7 (162.2, 43.5)
	96h	143.5 (153.4, 44.6)	159.2 (162.7, 30.3)	199.8 (208.8, 40.7)	189.7 (189.2, 40.5)	186.9 (182.4, 40.3)
1%	baseline	119.4 (120.0, 45.0)	122.8 (112.0, 45.9)	123.4 (122.4, 42.3)	123.8 (129.0, 39.5)	115.2 (114.2, 27.9)
	48h	155.1 (147.6, 51.1)	182.3 (183.8, 48.8)	187.1 (191.8, 37.3)	196.4 (189.4, 29.6)	183.1 (178.6, 45.4)
	96h	163.6 (173.4, 49.5)	176.5 (171.1, 31.0)	199.2 (203.8, 36.1)	198.8 (199.8, 25.2)	202.1 (191.6, 38.0)
2%	baseline	120.1 (120.8, 41.8)	113.8 (110.2, 40.3)	NA	NA	NA
	48h	177.7 (182.2, 48.4)	196.5 (197.0, 34.2)	NA	NA	NA
	96h	200.3 (193.8, 56.5)	194.5 (191.8, 37.9)	NA	NA	NA

A.27 SLS: MIXED EFFECTS MODEL ANALYSIS OF ΔE

Site	Time	2 vs 1	3 vs 2	3 vs 1	4 vs 5	4 vs 2	4 vs 1
Control	48 h	NS [2]	NS	NS	NS	NS	NS
	96 h	NS	NS	NS	NS	NS	NS
SW [1]	48 h	NS	NS	NS	NS	NS	NS
	96 h	NS	NS	NS	NS	NS	NS
0.0625%	48 h	0.09	NS	NS	NS	NS	NS
	96 h	NS	NS	NS	NS	NS	NS
0.125%	48 h	0.05	NS	NS	NS	NS	NS
	96 h	NS	NS	NS	NS	NS	NS
0.25%	48 h	NS	NS	NS	NS	NS	NS
	96 h	NS	NS	NS	NS	NS	NS
0.5%	48 h	NS	NS	NS	NS	NS	NS
	96 h	NS	NS	0,008	NS	NS	NS
1%	48 h	NS	NS	NS	NS	0,08	0,04
	96 h	NS	NS	NS	NS	NS	NS
2%	48 h	0,08	NA [3]	NA	NA	NA	NA
	96 h	NS	NA	NA	NA	NA	NA

[1]sterile water; [2]not significant; [3]not applicable

A.28 SLS: MEAN TEWL READINGS

Site	Time	Group 1 Mean (med*, SD)	Group 2 Mean (med, SD)	Group 3 Mean (med, SD)	Group 4 Mean (med, SD)	Group 5 Mean (med, SD)
Control	baseline	13.3 (8.0, 22.0)	8.0 (7.2, 3.5)	5.2 (4.8, 2.8)	5.4 (5.8, 2.7)	6.2 (5.6, 2.7)
	48h	14.1 (9.2, 21.1)	7.6 (7.1, 3.5)	4.9 (5.1, 2.3)	5.4 (4.8, 2.5)	5.9 (5.6, 2.4)
	96h	14.0 (7.9, 23.0)	6.5 (5.4, 3.4)	4.5 (4.5, 2.1)	4.9 (4.6, 2.8)	5.2 (5.0, 2.4)
SW[§]	baseline	14.3 (9.3, 21.5)	9.0 (8.1, 4.2)	6.1 (4.3, 4.3)	6.4 (7.0, 3.1)	7.1 (6.1, 2.9)
	48h	22.3 (17.7, 23.3)	16.3 (15.8, 7.2)	15.2 (16.7, 4.4)	15.9 (16.3, 4.4)	15.8 (15.5, 3.5)
	96h	15.3 (9.0, 23.1)	9.2 (7.9, 4.6)	5.8 (5.6, 3.0)	6.9 (7.1, 3.3)	7.0 (7.5, 3.2)
0.0625%	baseline	14.0 (9.6, 20.2)	8.1 (7.2, 4.0)	5.2 (4.6, 3.1)	6.1 (6.0, 2.7)	6.3 (5.1, 3.0)
	48h	24.7 (20.7, 17.9)	22.1 (21.4, 7.2)	19.1 (18.2, 4.6)	19.9 (21.6, 5.4)	19.4 (18.5, 5.8)
	96h	16.3 (11.2, 20.9)	11.1 (10.9, 5.5)	9.0 (8.8, 3.4)	10.9 (9.8, 4.9)	10.5 (10.6, 4.5)
0.125%	baseline	14.4 (8.6, 21.0)	8.3 (6.8, 4.3)	5.8 (3.7, 4.4)	6.2 (6.1, 2.6)	7.7 (6.9, 4.6)
	48h	24.0 (19.9, 18.6)	22.9 (22.0, 5.9)	19.9 (19.6, 4.4)	20.6 (20.7, 4.5)	18.8 (18.1, 4.7)
	96h	17.8 (11.7, 21.3)	14.3 (14.3, 6.2)	12.1 (11.6, 4.9)	15.8 (16.5, 6.5)	13.5 (12.8, 4.4)
0.25%	baseline	14.2 (8.7, 21.2)	8.9 (8.4, 4.3)	5.6 (4.7, 3.8)	6.3 (5.6, 3.1)	7.5 (6.9, 3.7)
	48h	25.3 (19.1, 23.3)	22.4 (21.3, 5.5)	18.8 (19.9, 5.5)	18.7 (19.2, 4.9)	24.1 (21.7, 9.9)
	96h	21.5 (15.1, 21.9)	16.9 (16.4, 7.1)	14.8 (13.8, 7.6)	14.6 (14.1, 6.0)	15.0 (13.9, 5.9)
0.5%	baseline	13.9 (8.6, 21.4)	8.0 (7.4, 4.1)	5.3 (4.9, 3.4)	5.5 (5.8, 2.5)	7.2 (5.7, 4.9)
	48h	27.1 (21.9, 19.1)	27.1 (23.7, 10.5)	23.4 (21.8, 6.9)	26.1 (25.0, 5.3)	30.8 (30.5, 6.8)
	96h	21.9 (17.6, 21.5)	20.1 (20.8, 8.9)	17.5 (17.6, 7.8)	20.2 (21.7, 5.4)	18.5 (18.0, 5.1)
1%	baseline	14.8 (8.8, 21.5)	8.6 (7.8, 4.0)	5.1 (4.5, 2.7)	5.4 (6.0, 2.1)	7.1 (6.0, 3.3)
	48h	29.2 (23.1, 21.0)	36.6 (39.0, 13.8)	40.5 (34.4, 17.3)	38.1 (35.7, 10.4)	42.5 (43.1, 10.9)
	96h	23.5 (17.3, 21.6)	22.1 (24.8, 7.6)	22.7 (22.1, 8.2)	23.2 (23.6, 4.6)	25.8 (23.0, 14.0)
2%	baseline	15.0 (10.0, 21.7)	8.4 (7.7, 3.7)	NA	NA	NA
	48h	49.2 (36.8 (28.8)	50.0 (46.0, 22.7)	NA	NA	NA
	96h	37.3 (24.5, 26.3)	29.0 (26.3, 15.7)	NA	NA	NA

[§]sterile water; * median

A.29 SLS: LINEAR MIXED EFFECTS MODEL OF ΔTEWL

Site	Time	2 vs.1	3 vs.2	3 vs. 1	4 vs.5	4 vs.1
Control	48h	0.07	NS	NS	NS	NS
	96h	0.09	NS	NS	NS	NS
SW[1]	48h	NS[2]	NS	NS	NS	NS
	96h	NS	NS	NS	NS	NS
0.0625%	48h	0.08	NS	NS	NS	NS
	96h	NS	NS	NS	NS	0.03
0.125%	48h	0.007	0.005	NS	0.04	NS
	96h	0.05	NS	NS	0.05	0.002
0.25%	48h	NS	NS	NS	0.07	NS
	96h	NS	NS	NS	NS	NS
0.5%	48h	0.04	NS	NS	0.09	0.06
	96h	0.05	NS	NS	0.09	0.01
1%	48h	0.006	0.007	0.001	NS	0.005
	96h	0.03	0.04	0.002	NS	<0.001
2%	48h	NS	NA[3]	NA	NA	NA
	96h	NS	NA	NA	NA	NA

[1]sterile water; [2]not significant; [3]not applicable

A.30 SLS: VARIATION IN SKIN HYDRATION LEVELS (ΔCM)

	Time	Group 1 Mean (SD)	Group 2 Mean (SD)	Group 3 Mean (SD)	Group 4 Mean (SD)	Group 5 Mean (SD)
rol	48 h	2.6 (52.8)	18.3 (32.8)	28.7 (47.0)	31.7 (46.4)	38.2 (36.3)
	96 h	0.4 (21.9)	7.2 (54.9)	20.3 (42.3)	10.6 (43.5)	28.4 (29.1)
	48 h	-15.0 (68.9)	-11.4(66.6)	-23.6 (67.6)	-2.3 (49.6)	1.8 (48.9)
	96 h	6.6 (32.8)	30.2 (58.1)	26.1 (70.2)	9.5 (48.5)	41.5 (36.4)
25%	48 h	-15.7 (71.2)	-34.8 (56.8)	-31.2 (44.4)	-12.1 (44.7)	-30.3 (48.6)
	96 h	-0.9 (31.0)	30.9 (53.7)	56.8 (74.7)	30.9 (61.8)	47.2 (53.8)
5%	48 h	-20.6 (73.8)	-38.0 (58.0)	-22.8 (95.9)	-3.6 (33.7)	-22.2 (47.2)
	96 h	5.7 (32.2)	13.8 (40.8)	35.1 (77.6)	31.5 (41.1)	41.4 (69.4)
%	48 h	-14.8 (71.1)	-28.1 (54.9)	-12.7 (89.2)	1.4 (38.0)	56.6 (109.9)
	96 h	-2.8 (40.5)	-17.5 (52.9)	38.0 (81.9)	3.9 (38.2)	-11.1 (63.8)
	48 h	14.9 (57.9)	20.8 (81.0)	91.4 (116.3)	78.6 (91.8)	129.5 (201.2)
	96 h	-14.4 (51.2)	-33.2 (75.7)	-14.9 (45.5)	-16.0 (63.1)	-42.9 (88.6)
	48 h	32.8 (66.9)	93.0 (140.5)	235.7 (275.1)	225.8 (186.4)	322.4 (400.9)
	96 h	-17.2 (41.5)	-52.2 (72.8)	-58.8 (75.8)	-52.2 (36.2)	-71.7 (79.2)
	48 h	329.9 (499.7)	236.2 (227.3)	NA	NA	NA
	96 h	-26.9 (65.9)	-72 (65.7)	NA	NA	NA

[1]sterile water

A.31 SLS: LINEAR MIXED EFFECTS MODEL ANALYSIS OF ΔCM

Site	Time	2 vs.1	3 vs. 2	3 vs. 1	4 vs.5	4 vs. 1
Control	48h	NS	NS	NS	NS	NS
	96h	NS	NS	NS	NS	NS
SW	48h	NS	NS	NS	NS	NS
	96h	NS	NS	NS	NS	NS
0.0625%	48h	NS	NS	NS	NS	NS
	96h	0.05	NS	0,01	NS	NS
0.125%	48h	NS	NS	NS	NS	NS
	96h	NS	NS	NS	NS	NS
0.25%	48h	NS	NS	NS	0.06	NS
	96h	NS	NS	0.01	NS	NS
0.5%	48h	NS	NS	0.005	NS	0.01
	96h	NS	NS	NS	NS	NS
1%	48h	NS	NS	0.001	NS	<0.001
	96h	NS	NS	NS	NS	NS
2%	48h	NS	NA	NA	NA	NA
	96h	0,06	NA	NA	NA	NA

Appendix B



Fig. B1 – Erythema meter and tewameter.



Fig. B2 – Marking the test sites.



Fig. B3 – Securing the test strips.



Fig. B4 – DNCB challenge sites at 48 hours in a transplant patient with SCCs.



Fig. B5 – DNCB challenge sites at 48 hours in a transplant patient without SCCs.



Fig. B6 – DNCB challenge sites at 96 hours in a transplant patient without SCCs.



Fig. B7 – DNCB challenge sites at 48 hours in a non-immunosuppressed patient with SCC.



Fig. B8 – DNCB challenge sites at 96 hours in a non-immunosuppressed patient with SCC.



Fig. B9 – DNCB challenge sites at 48 hours in a normal control.



Fig. B10 – DNCB challenge sites at 96h in a normal control.



Fig. B11 – Recall antigen testing at 48 hours (normal control).



Fig. B12 – Dithranol test sites at 96 hours in a transplant patient with SCCs.

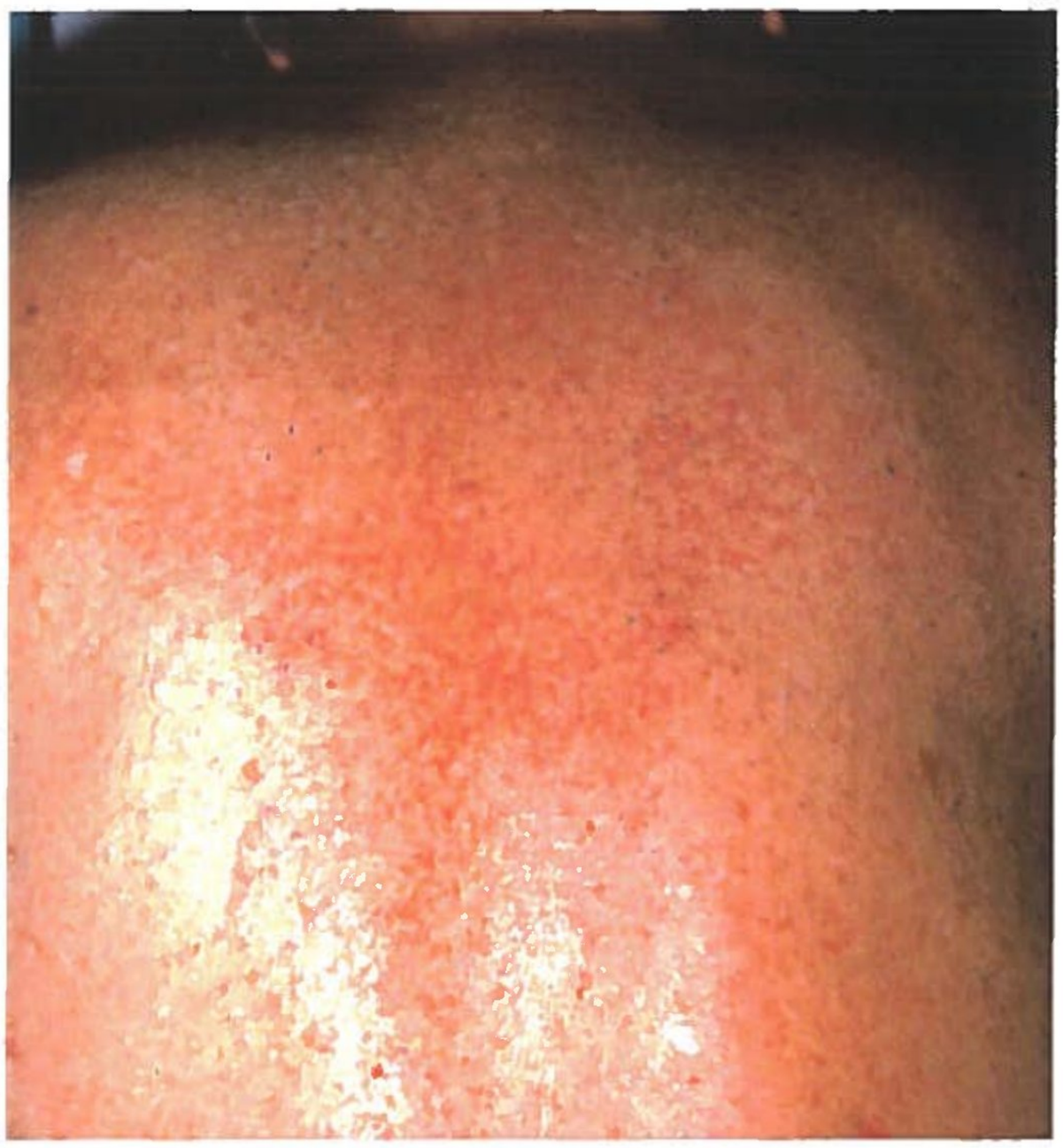


Fig. B13 – SLS test sites at 96 hours in a transplant patient with SCCs.



Fig. B14 – Dithranol and SLS test sites at 96 hours in a transplant patient without SCCs.



Fig. B15 – Dithranol test sites at 96 hours in a normal control



Fig. B16 – SLS test sites at 96 hours in a normal control

Bibliography

Aberer, W., Stingl, L., Pogantsch, S. & et al. (1984). Effect of glucocorticosteroids on epidermal cell-induced immune responses. *J Immunol*, **133(2),** 792-7.

Alamartine, E. & Bertoux, F. (1995). Tumours in kidney-transplanted patients: a comprehensive one-centre study. *Transplantation Proc*, **27(2),** 1761-3.

Ananthaswamy, H.N. & Pierceall, W.E. (1990). Molecular mechanisms of untraviolet radiation carcinogenesis. *Photochemistry Photobiology*, **52,** 1119-1136.

Ashworth, J., Booker, J. & Breathnach, S.M. (1988). Effects of topical corticosteroid therapy on Langerhans cell antigen presenting function in human skin. *Br J Dermatol*, **118(4),** 457-69.

Astori, G., Lavergne, D., Benton, C. & et al. (1998). Human papillomaviruses are commonly found in normal skin of immunocompetent hosts. *J Invest Dermatol*, **110(5),** 752-5.

Aubry, F. & MacGibbon, B. (1985). Risk factors of squamous cell carcinoma of the skin. A case-control study in the Montreal region. *Cancer*, **55(4),** 907-11.

Baadsgaard, O., Lisby, S., Wantzin, S. & et al. (1989). Rapid recovery of Langerhans cells alloreactivity, without induction of autoreactivity, after in vivo ultraviolet A, but not ultraviolet B exposure of human skin. *J Immunol*, **142,** 4213-18.

Barranco, V.P., Olson, R.I. & Everett, M.A. (1970). Response of actinic keratoses to topical vitamin A acid. *Cutis* , **6**, 681.

Belsito, D.V., Flotte, T.J., Lim, H.W. & et al. (1982). Effect of glucocorticoids on epidermal Langerhans cells. *J Exp Med*, **155(1),** 291-302.

Benson, P.M., Pessoa, C.M. & et al. (1988). Basal cell carcinoma arising in chronic lymphoedema. *J Dermatol Surg Oncol*, **14,** 781-3.

Benton, E.C., Shahidullah, H. & Hunter, J.A. (1992). Human papillomavirus in the immunosuppressed. *Papillomavirus Rep*, **3,** 23-6.

Berardesca, E. & Maibach, H.I. (1988). Bioengineering and the patch test. *Contact Dermatitis*, **18,** 3-9.

Berger, M.R. (1991). Animal models of cancer caused by immunosuppression. In *Cancer in organ transplant recipients*, Schmahl, D. & Penn, I. (eds) pp. 3-17. Springer-Verlag: Berlin Heidelberg.

Bergfelt, L. (1993). Langerhans cells, immunomodulation and skin lesions. A quantitative, morphological and clinical study. *Acta Derm Venereol Suppl (Stockh)*, **180,** 1-37.

Berman, B., France, D.S., Martinelli, G.P. & et al. (1983). Modulation of expression of epidermal Langerhans cell properties following in situ exposure to glucocorticosteroids. *J Invest Dermatol*, **80(3),** 168-71.

Blohme, I. & Larko, O. (1990). Skin lesions in renal transplant patients after 10-23 years of immunosuppressive therapy. *Acta Derm Venereol (Stockh)* , **70**, 491-494.

Bordea, C., Wojnarowska, F. & Morris, P.J. (1999). Multiple cutaneous malignancies arising in limbs with signs of lymphatic insufficiency in transplant patients. *Br J Plast Surg*, **52,** 619-622.

Bos, J.D. & Kapsenberg, M.L. (1986). The skin immune system (SIS): its cellular constituents and their interactions. *Immunol Today*, **7,** 235.

Bos, J.D., Das, P.K. & Kapsenberg, M.L. (1997). Skin immune system. In *Skin Immunology*, Bos, J.D. (ed). CRC Press: Boca Raton, Florida.

Bouwes Bavinck, J., Vermeer, B.J., Van der Woude, F. & et al. (1991). Relation between skin cancer and HLA antigens in renal transplant recipients. *N Engl J Med*, **325(12),** 843-8.

Bouwes Bavinck, J.N., Claas, F.H.J., Hardie, D.R. & et al. (1997). Relation between HLA antigens and skin cancer in renal transplant recipients in Queensland, Australia. *J Invest Dermatol*, **108(5)**, 708-11.

Bouwes Bavinck, J.N., Hardie, D.R., Green, A. & et al. (1996). The risk of skin cancer in transplant patients in Queensland, Australia. *Transplantation* , **61**, 715-21.

Bouwes Bavinck, J.N., Koote, A.M.M., Van der Woude, F.J. & et al. (1990). HLA A11-associated resistance to skin cancer in renal transplant recipients [letter]. *N Engl J Med*, **323(19)**, 1350.

Bouwes Bavinck, J.N., Kootte, A.M.M., Van der Woude, F.J. & et al. (1991b). On a possible protective effect of HLA A11 against skin cancer and keratotic skin lesions in renal transplant recipients. *J Invest Dermatol*, **97(2)**, 269-72.

Bouwes Bavinck, J.N., Tieben, L.M., Woude, F.K.V.D. & et al. (1995). Prevention of skin cancer and reduction of keratotic skin lesions during acitretin therapy in renal

transplant recipients; a double-blind, placebo-controlled study. *J Clin Oncol* , **18,** 1933-38.

Braathen, L.R. & Thorsby, E. (1980). Studies on human epidermal Langerhans cells. I. Allo-activating and antigen presenting capacity. *Scand J Immunol*, **11(4),** 401-8.

Brinkman, M.N.B., Huizinga, T.W.J., Breedveld, F.C. & Verweij, C.L. (1996). Allele-specific quantification of TNF alpha transcripts in rheumatoid arthritis. *Arthritis Rheum*, **39 (suppl)**, S224.

Brown, J.H., Hutchinson, T., Kelly, A.M.T. & et al. (1988). Dermatologic lesions in a transplant population. *Transplantation*, **46(4),** 530-2.

Bunce, M., O'Neill, C., Barnardo, M. & al, e. (1995). Phototyping. Comprehensive DNA typing for HLA-A,B, C, DRB1, DRB3, DRB4, DRB5 and DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). *Tissue Antigens*, **50,** 23-31.

Cerimele, D., Contu, L., Carcassi, C. & et al. (1988). HLA and multiple skin carcinomas. *Dermatologica*, **176(4)**, 176-81.

Chaterjee, S.N. (1985). Immunologic monitoring with recall antigen skin tests in uraemic patients and renal transplant patients. *Dialysis Transplant*, **14,** 572-7.

Chouchane, L., Ahmed, S.B., Baccouche, S. & Remadi, S. (1997). Polymorphisms in the tumour necrosis factor-alpha promoter region and in the heat shock protein 70 genes associated with malignant tumours. *Cancer*, **80**, 1489-1496.

Cohen, E.B., Komorowski, R.A. & Clowry, L.C. (1987). Cutaneous complications in renal transplant recipients. *Am J Clin Pathol* , **88(1),** 32-7.

Crowley, N.J., Darrow, T.L., Quinn-Allen, M.A. & al, e. (1991). MHC-restricted recognition of autologous melanoma by tumour-specific cytotoxic T cells: evidence for restriction by a dominant HLA allele. *J Immunol*, **146,** 1692-9.

Cunningham, T.J., Daut, D., Wolfgang, D.E. & al, e. (1976). Correlation of DNCB-induced delayed cutaneous hypersensitivity reaction and the course of disease in patients with recurrent breast cancer. *Cancer*, **37,** 1696-1700.

Curson, C. & Weedon, D. (1979). Spotaneous regression in basal cell carcinomas. *J Cutan Pathol*, **6,** 432-7.

Czarnecki, D., Lewis, A., Nicholson, I. & et al. (1991). Multiple basal cell carcinomas and HLA frequencies in Southern Australia. *J Am Acad Dermatol*, *24(4)*, 559-61.

Czarnecki, D., Mar, A. & Kulinskaya, E. (1996). Cell-mediated immunity of patients who have had basal cell carcinomas. *Acta Derm Venereol (Stockh)*, **76,** 28-30.

Czarnecki, D., Nicholson, I., Tait, B. & Nash, C. (1993). HLA DR4 is associated with the development of multiple basal cell carcinomas and malignant melanoma. *Dermatology*, **187(1)**, 16-8.

Czarnecki, D., Tait, B., Nicholson, I. & Lewis, A. (1994). Multiple non-melanoma skin cancer: evidence that different MHC genes are associated with different cancers. *Dermatology*, **188,** 88-90.

Czarnecki, D., Watkins, F., Leahy, S. & et al. (1992). Skin cancers and HLA frequencies in renal transplant recipients. *Dermatology*, **185(1)**, 9-11.

D'Alfonso, S.M.P. (1996). A new polymorphism in the TNFA gene (abstract). *Hum Immunol*, **47**, 65.

D'Alfonso, S.R.P.M. (1994). A polymorphic variation in a putative regulatory box of the TNFA promoter region. *Immunogenetics*, **39**, 150-154.

Dantal, J., Hourmant, M., Cantarovich, D. & et al. (1998). Effects of long-term immunosuppression in kidney-graft recipients on cancer incidence: randomised comparison of two cyclosporine regimens. *Lancet*, **351(9103),** 623-8.

de Berker, D., Ibbotson, S., Simpson, N.B. & al, e. (1995). Reduced experimental contact sensitivity in squamous cell but not basal cell carcinoma of skin. *Lancet*, **345,** 425-6.

de Boer, E.M. & Bruynzeel, D.P. (1996). Patch test: Evaluation by instrumental methods. *Clin Dermatol*, **14,** 41-50.

de Villiers, E.-M., Lavergne, D., McLaren, K. & Benton, E.C. (1997). Prevailing papillomavirus types in non-melanoma carcinomas of the skin in renal allograft recipients. *Int J Cancer*, **73(3),** 356-61.

Diffey, B.L., Oliver, R.J. & Farr, P.M. (1984). A portable instrument for quantifying erythema induced by ultraviolet radiation. *Br J Dermatol*, **111(6),** 663-72.

Disney, A.P.S., Russ, G.R., Walker, R. & Sheil, A.G.R. (1997). ANZDATA Registry Report 1997. Australia and New Zealand Transplant Registry: Adelaide, South Australia.

Doak, P.B., Montgomerie, J.L., North, J.D.K. & Smith, F. (1968). Reticulum cell sarcoma after renal transplantation and azathioprine and prednisone therapy. *Br Med J* , **4**, 746-8.

Dobson, A.J. (1983). *Statistical Modelling*. Chapman&Hall: London.

Dupont, E., Schandene, L., Denys, C. & et al. (1986). Differential in-vitro actions of cyclosporin, methylprednisolone and 6-mercaptopurine: implications for drugs' influence on lymphocyte activation mechanisms. *Clin Immunol Immunopathol*, **40,** 422.

Dupuy, P., Bagot, M., MIchel, L. & et al. (1991). Cyclosporine A inhibits the antigen-presenting functions of freshly isolated human Langerhans cells in vitro. *J Invest Dermatol*, **96(4),** 408-13.

Epstein, J.I. & Mendelsohn, G. (1984). Squamous cell carcinoma of the foot arising in association with longstanding verrucous hyperplasia in a patient with congenital lymphoedema. *Cancer*, **54,** 943-7.

Fabo, E.C.D. & Noonan, F.P. (1983). Mechanism of immune suppression by ultraviolet radiation *in vivo*. I. Evidence for the existence of a unique photoreceptor in skin and its role in photoimmunology. *J Exp Med*, **157,** 84-98.

Fabo, E.C.D., Noonan, F.P., Fisher, M. & al, e. (1983). Further evidence that the photoreceptor mediating UV-induced systemic immune suppression is urocanic acid. *J Invest Dermatol*, **80,** 319 (Abstr.).

Farr, P.M. & Diffey, B.L. (1984). Quantitative studies on cutaneous erythema induced by ultraviolet radiation. *Br J Dermatol*, **111(6),** 673-82.

Ferrandiz, C., Fuente, M.J., Ribera, M. & et al. (1995). Epidermal dysplasia and neoplasia in kidney transplant recipients. *J Am Acad Dermatol* , **33**, 590-6.

Franchimont, C., Pierard, G.E., Van Cauwenberge, D. & al, e. (1982). Episodic progression and regression of basal cell carcinoma. *Br J Dermatol*, **106,** 305-10.

Friedman, P.S., Moss, C., Shuster, S. & Simpson, J.M. (1989). Sex differencies in susceptibility to development of contact hypersensitivity to dinitrochlorobenzene. *Br J Dermatol*, **122,** 29-31.

Furue, M. & Katz, S. (1988). The effect of cyclosporine on epidermal cells. I. Cyclosporine inhibits accessory cell functions of epidermal Langerhans cells in vitro. *J Immunol*, **140,** 4139-43.

Gabel, H., Jontell, M., Ohman, C. & Brynger, H. (1987). Epidermal Langerhans cells in the early phase of immunosuppression. *Transplant Proc*, **19(1 Pt 2),** 1205-6.

Gaya, S.B.M., Rees, A.J., Lechler, R.I. & et al. (1995). Malignant disease in patients with long-term renal transplants. *Transplantation* , **59(12)**, 1705-9.

Gibson, G.E., O'Grady, A., Kay, E.W. & Murphy, G.M. (1998). Low-dose retinoid therapy for chemoprophylaxis of skin cancer in renal transplant recipient. *J Europ Acad Dermatol Venereol* , **10**, 42-47.

Glover, M., Cerio, R., Corbett, M. & et al. (1995). Cutaneous squamoproliferative lesions in renal transplant recipients. *Am J Dermatopathol* , **17(6)**, 551-54.

Glover, M.J., Deeks, J., Kwan, J. & et al. (1997). Is cyclosporine associated with an increased risk of skin cancer in renal transplant patients (abstract). *Br J Dermatol*, 286.

Glover, M.T., Bodmer, J., Bodmer, W. & et al. (1993). HLA antigen frequencies in renal transplant recipients and non-immunosuppressed patients with nonmelanoma skin cancer. *Eur J Cancer*, **29A(4),** 520-4.

Glover, M.T., Niranjan, N., Kwan, J.T.C. & et al. (1994). Non-melanoma skin cancer in renal transplant recipients: the extent of the problem and a strategy for management. *Br J Plast Surg* , **47(2)**, 86-9.

Goldblum, O.M., Kraus, E. & Bronner, A.K. (1985). Pseudo-Kaposi's sarcoma of the hand associated with an acquired, iatrogenic arteriovenous fistula. *Arch Dermatol*, **121,** 1038-40.

Gooding, L.R. & O'Connell, K.A. (1983). Recognition by cytotoxic T lymphocytes of cells expressing fragments of the SV40 tumour antigen. *J Immunol*, **131,** 2580-6.

Gooptu, C., Woollons, A., Ross, J. & et al. (1997). Merkel cell carcinoma arising after therapeutic immunouppression. *Br J Dermatol* , **137**, 637-41.

Grabbe, S., Bruvers, S., Gallo, R.I. & et al. (1991). Tumour antigen presentation by murine epidermal cells. *J Immunol*, **146(10),** 3656-61.

Greenberg, P.D., Klarnet, J.P., Kern, D.E. & Cheever, M.A. (1988). Therapy of disseminated tumours by adoptive transfer of specifically immune T cells. *Progr Exp Tumour Res*, **32,** 104-27.

Gupta, A.K., Cardella, C.J. & Haberman, H.F. (1986). Cutaneous malignant neoplasms in patients with renal transplants. *Arch Dermatol* , **122**, 1288-1293.

Habets, J.M., Tank, B., Vuzevski, V.D. & al, e. (1988). Characterisation of the mononuclear cell infiltrate in basal cell carcinoma: a predominantely T cell-mediated immune response with minor participation of Leu-7+ (natural killer) cells and Leu-14+ (B) cells. *J Invest Dermatol*, **90,** 289-292.

Habets, J.M.W., Tank, B. & van Joost, T. (1989). Characterization of the mononuclear infiltrate in Bowne's disease (squamous cell carcinoma in situ). Evidence for a T cell-mediated anti-tumour response. *Virchows Archiv A Pathol Anat Histopathol*, **415(2),** 125-30.

Halpern, A.C. & Altman, J.F. (1999). Genetic predisposition to skin cancer. *Curr Opin Oncol*, **11,** 132-8.

Hamann, A., Mantzoros, C., Vidal-Puig, A. & flier, J.S. (1995). Genetic varibility in the TNF-alpha promoter is not associated with type II diabetes mellitus (NIDDM). *Biochem Biophys Res Commun*, **211**, 833-839.

Hardie, I.R. (1995). Skin cancer in transplant recipients. *Transpl Rev* , **9(1),** 1-16.

Hardie, I.R., Strong, R.W., Hartley, L.C.J. & et al. (1980). Skin cancer in caucasian renal allograft recipients living in a subtropical climate. *Surgery* , **87**, 177.

Hartevelt, M.M., Bouwes Bavinck, J.N., Kootte, A.M.M. & et al. (1990). Incidence of skin cancer after transplantation in The Netherlands. *Transplantation* , **49(3)**, 506-9.

Heagerty, A., Smith, A., English, J., Lear, J. & et. al. (1996). Susceptibility to multiple cutaneous basal cell carcinomas: significant interactions between glutathione S-transferase GSTM1 genotype, skin type and male gender. *Br J Cancer*, **73,** 44-48.

Heagerty, A.H.M., Fitzgerald, D., Smith, A. & et al. (1994). Glutathione S-transferase GSTM1 phenotype and protection against cutaneous tumours. *Lancet*, **343,** 266-268.

Helland, A., Langerod, A., Johnsen, H. & et al. (1998). p53 polymorphism and risk of cervical cancer. *Nature*, **396,** 530-531.

Hepburn, D.J., Divakar, D., Bailey, R.R. & et al. (1994). Cutaneous manifestations of renal transplantation in a New Zealand population. *N Z Med J*, **107(991),** 497-9.

Heylings, J.R., Clowes, H.M., Cumberbatch, M. & al, e. (1996). Sensitization to 2,4-dinitrochlorobenzene: influence of vehicle on absorption and lymph node activation. *Toxicology*, **109,** 57-65.

Hiesse, C., Larue, J.R., Kriaa, F. & et al. (1995). Incidence and type of malignancies occuring after renal transplantation in conventionally and in cyclosporine-treated recipients: single-centre analysis of a 20-year period in 1600 patients. *Transplantation Proc*, **27(4),** 2450-51.

Higuchi, T., Seki, N., Yamada, A. & al, e. (1998). Polymorphism of the 5'-flankin region of the human tumour necrosis factor (TNF)-alpha gene in Japanese. *Tissue antigens*, **51**, 605-612.

Hildesheim, A., Schiffman, M., Brinton, L.A. & et al. (1998). p53 polymorphism and risk of cervical cancer. *Nature*, **396,** 532.

Holgate, S.T. & Church, M.K. (1993). Eczema and contact dermatitis-pathophysiology. In *Allergy* pp. 23.1-23.10. Gower Medical Publishing.

Howley, P.M. (1996). Papillomavirinae: The viruses and their replication. In *Fields Virology*, Fields, B.N., Knipe, D.M., Howley, P.M. & al (eds) pp. 2045-76. Lippincott-Raven: Philadelphia.

Hunt, M.J., Halliday, G.M., Weedon, D. & et al. (1994). Regression in basal cell carcinoma: an immunohistochemical analysis. *Br J Dermatol*, **130(1),** 1-8.

Janeway, C.A. & Travers, P. (1996a). *Immunobiology*. Current Biology Ltd

Janeway, C.A.J. & Travers, P. (1996b). Antigen recognition by T lymphocytes. In *Immunobiology; The Immune system in health and disease*, Janeway, C.A.J. & Travers, P. (eds) pp. 4:1-4:49. Current Biology Ltd/ Garland Publishing Inc.

Jensen, M.K. (1967). Chromosome studies in patients treated with azathioprine and amethopterin. *Acta Med Scand*, **182,** 445.

Josefsson, A.M., Magnusson, P.K., Ylitalo, N. & et al. (1998). p53 polymorphism and risk of cervical cancer. *Nature*, **396,** 531-532.

Karagas, M.R., Stukel, T.A., Greenberg, E.R. & et al. (1992). Risk of subsequent basal cell carcinoma and squamous cell carcinoma of the skin among patients with prior skin cancer. *J A M A*, **267 (24),** 3305-10.

Kast, W.M., Offringa, R. & Peters, P.J. (1989). Eradication of adenovirus E1-induced tumours by E1A-specific cytotoxic T lymhpocytes. *Cell*, **59,** 603-14.

Kastan, M.B., Onyekwere, O., Sidransky, D. & et al. (1991). Participation of p53 protein in the cellular response to DNA damage. *Cancer Research*, **51,** 6304-6311.

Kelly, G., Scheibner, A., Murray, E. & et al. (1987). T6+ and HLA-DR+ cell numbers in epidermis of immunosuppressed renal transplant recipients. *J Cutan Pathol*, **14(4),** 202-6.

Kelly, G.E., Sheil, A.G.R., Wass, J. & al, e. (1986). Effects of ultraviolet irradiation and immunosuppressive therapy on mouse epidermal cell kinetics. *Br J Dermatol*, **114**.

Kelly, J.W., Sabto, J., Gurr, F.W. & et al. (1991). Retinoids to prevent skin cancer in organ transplant recipients. *Lancet* , **2**, 1407.

Kinlen, L.J., Sheil, A.G.R., Peto, J. & Doll, R. (1979). Collaborative United Kingdom-Australasian study of cancer in patients treated with immunosuppressive drugs. *Br Med J* , 2(6203), 1461-6.

Kirkham, N. (1997). Tumours and cysts of the epidermis. In *Lever's Histopathology of the Skin* , Elder, D., Elenitsas, R. & Jaworsky, C. (eds) pp. 685-746. Lippincott-Raven.

Knicker, W.T., Anderson, C.T., McBryde, J.L. & al, e. (1984). Multitest CMI for standardised measurement of delayed cutaneous hypersensitivity and cell-mediated immunity. Normal values and proposed scoring system for healthy adults in the USA. *Ann Allergy*, **52,** 43-49.

Knight, J.C., Udalova, I., Hill, A.V.S. & al, e. (1999). A polymorphism that affects OCT-1 binding to the *TNF* promoter region is associated with severe malaria. *Nature Genetics*, **22**, 145-150.

Knudson, A.G. (1993). Antioncogenes and human cancer. *Proc Nat Acad Sci USA*, **90,** 10914-21.

Ko, C.B., Walton, S., Keczkes, K. & et al. (1994). The emerging epidemic of skin cancer. *Br J Dermatol* , **130**, 269-72.

Lampert, I.A. (1984). Expression of HLA-DR (Ia like) antigen on epidermal keratinocytes in human dermatoses. *Clin Exp Immunol*, **57,** 93.

Landthaler, M., Stolz, W., Eckert, F. & et al. (1989). Pseudo-Kaposi's sarcoma occuring after placement of arteriovenous shunt. *J Am Acad Dermatol*, **21,** 499-505.

Lane, D.P. (1994). The regulation of p53 function: Steiner award lecture. *International Journal of Cancer*, **57,** 623-627.

Larsen, C.P., Ritchie, S.C., Hendrix, R. & et al. (1994). Regulation of immunostimulatory function and costimulatory molecule (B7-1 and B7-2) expression on murine dendritic cells. *J Immunol*, **152(11),** 5208-19.

Larsen, C.P., Steinman, R.M., Witmer-Pack, M. & et al. (1990). Migration and maturation of Langerhans cells in skin transplants and explants. *J Exp Med*, **172(5),** 1483-93.

Lear, J.T., Heagerty, A.H., Smith, A. & et al. (1996). Multiple cutaneous basal cell carcinomas: glutathione S-transferase (GSTM1, GSTT1) and cytochrome P450

(CYP2D6, CYP1A1) polymorphisms influence tumour numbers and accrual. *Carcinogenesis*, **17,** 1891-1896.

Lear, J.T., Heagerty, A.H.M., Smith, A. & et. al. (1997). Truncal site and detoxifying enzyme polymorphisms significantly reduce time to presentation of next cutaneous basal cell carcinoma. *Carcinogenesis*, **18,** 1499-1503.

Lear, J.T., Smith, A.G., Strange, R.C. & Fryer, A.A. (2000). Detoxifying enzyme genotypes and susceptibility to cutaneous malignancy. *British Journal of Dermatology*, **142,** 8-15.

Liddington, M., Richardson, A.J., Higgins, R.M. & et al. (1989). Skin cancer in renal transplant recipients. *Br J Surg* , **76(10),** 1002-5.

Lodge, P. (1995). Malignancy: incidence and impact. In *International Congress and Symposium Series*, Vol. 217.

London, N.J., Farmery, S.M., Will, E.J. & et al. (1995). Risk of neoplasia in renal transplant patients. *The Lancet*, **346(8972),** 403-6.

Lotem, M., Tamir, G., Loven, G. & et al. (1994). Multiple basal cell carcinomas of the leg after recurrent erysipelas and chronic lymphoedema. *J Am Acad Dermatol*, **31,** 812-13.

Lowe, S.W., Schmitt, E.M., Smith, E.W. & et. al. (1993). p53 required for radiation-induced apoptosis in mouse thymocytes. *Nature*, **362,** 847-849.

Lutzner, M.A., Orth, G., Dutronquay, V. & al, e. (1983). Detection of human papillomavirus type 5 DNA in skin cancers of an immunosuppressed renal allograft recipient. *Lancet*, **2,** 422.

MacKie, R.M. (1998a). Epidermal skin tumours. In *Textbook of dermatology* , Champion, R.H., Burton, J.L., Burns, D.A. & Breathnach, S.M. (eds), Vol. 2. pp. 1651-94. Blackwells Science.

Mandel, M.A. & Kiehn, C.L. (1974). The prognostic significance of delayed cutaneous reactivity in head and neck cancer patients. *Plast Reconstr Surg*, **53,** 72-76.

Marcussen, J.A. & Tyden, G. (1988). Acne conglobata in transplant patients treated with isotretinoin. *Br J Dermatol* , **118**, 310.

Marshall, S.E., Bordea, C., Haldar, N.A., Mulligan, C.G. & al, e. (2000a). Glutathione S-transferase polymorphisms and skin cancers after renal transplantation. *Kidney International*, **58,** 2186-93.

Marshall, S.E., Bordea, C., Wojnarowska, F. & et al. (2000b). p53 codon polymorphism and susceptibility to skin cancer after renal transplantation. *Transplantation*, **69(5),** 994-996.

Marshall, V. (1974). Premalignant and malignant skin tumours in immunosuppressed patients. *Transplantation* , **17**, 272.

Martin, C.D., Rubini, M. & Rosen, V.J. (1965). Cadaveric renal transplantation with inadvertent transplantation of carcinoma. *J A M A* , **192**, 752.

McGregor, J.M., Barker, J.N.W.N. & MacDonald, D.M. (1991). The development of excess numbers of melanocytic naevi in an immunosuppressed identical twin. *Clin Exp Dermatol* , **16**, 131-2.

McGregor, J.M., Berkhout, R.J., Rozycka, M. & et al. (1997). p53 mutations implicate sunlight in post-transplant skin cancer irrespective of human papilloma virus status. *Oncogene*, **15(14),** 1737-1740.

McGregor, J.M., Reddi, G., MacDonald, D. & et al. (1992). HLA A11 in renal allograft recipients with skin cancer [letter]. *J Invest Dermatol*, **98(2),** 261-2.

McKee, P.H. (1996). Tumours of the epidermal appendages in *Pathology of the skin with clinical correlations*, pp.15.1-15.87, Mosby-Wolfe.

McKenna, D.B. & Murphy, G.M. (1999). Skin cancer chemoprophylaxis in renal transplant recipients: 5 years of experience using low-dose acitretin. *B J Dermatol* , **140**, 656-60.

McLelland, J., Rees, A., Williams, G. & et al. (1988). The incidence of immunosuppression-related skin disease in long-term transplant patients. *Transplantation*, **46(6),** 871-4.

McNutt, N.S., Saenz-Santamaria, C., Volkenandt, M. & et al. (1994). Abnormalities of p53 protein expression in cutaneous disorders. *Archives of Dermatology*, **130,** 225-232.

McPhaul, J.J., McIntosh, D.A. & Hall, W. (1965). Tissue transplantation still vexes. *N Engl J Med* , **272**, 105.

Melief, C.J.M. & Kast, W.M. (1991). Cytotoxic T lymphocyte therapy therapy of cancer and tumour escape mechanisms. *Semin Cancer Biol*, **2,** 347-54.

Memon, A.A. & Friedman, P.S. (1996). Studies on the reproducibility of allergic contact dermatitis. *Br J Dermatol*, **134(2),** 208-14.

Merimsky, O. & Chaitchik, S. (1992). Kaposi's sarcoma on a lymphoedematous arm following radical mastectomy. *Tumori*, **78,** 407-8.

Montgomery, D.C. (1984). *Design and analysis of experiments*. John Wiley & sons: New York.

Morris, D.L., Hersh, E.M., Hsi, B.P. & al, e. (1979). Recall antigen delayed-type hypersensitivity skin testing in melanoma and acte leukaemia patients and their associates. *Cancer Research*, **39,** 219-226.

Morris, P.J. (1988). Cyclosporine. In *Renal Transplantation: Principles and Practice* , Morris, P.J. (ed) pp. 179-201. W B Saunders.

Morris, P.J. (1988). Cyclosporine. In *Renal Transplantation: Principles and Practice*, Morris, P.J. (ed) pp. 179-201. W B Saunders.

Mullen, D.L., Silverberg, S.G., Penn, I. & Hammond, W.S. (1976). Squamous cell carcinoma of the skin and lip in renal homograft recipients. *Cancer* , 37, 729-34.

Nagano, T., Ueda, M. & Ichihashi, M. (1993). Expression of p53 is an early event in ultraviolet induced cutaneous squamous cell carcinogenesis. *Arch Dermatol*, **129,** 1157-1161.

Old, L.J. (1985). Tumour necrosis factor (TNF). *Science*, **230**, 630-632.

Orth, G. (1987). The Papillomaviruses. In *The Papovaviridae*, Salzman, N.P. & Howley, P.M. (eds), Vol. 2. pp. 199-243. Plenum Press: New York.

Patel, A., Halliday, G.M., Cooke, B.E. & Barnetson, R.S. (1994). Evidence that regression in keratoacanthoma is immunologically mediated: a comparison with squamous cell carcinoma. *Br J Dermatol*, **131(6),** 789-98.

Penn, I. (1994a). Occurence of cancers in immunosuppressed organ transplant recipients. In *Clinical Transplants* , Cecka, T.a. (ed) pp. 99-109. UCLA Tissue Typing Laboratory: Los Angeles.

Penn, I. (1994b). Posttransplant malignancies in pediatric organ transplant recipients. *Transplant Proc* , **26**, 2763-5.

Penn, I., Hammond, W., Brettschneider, L. & Starzl, T.E. (1969). Malignant lymphomas in transplantation patients. *Transplant Proc* , **1**, 106-12.

Petzelbauer, P. & Wolff, K. (1992). Effects of cyclosporine A on resident and passenger immune cells of normal human skin and UV-induced erythema reactions. *Br J Dermatol*, **127(6),** 560-5.

Pfister, H. (1992). *Sem Cancer Biol*, **3,** 263-71.

Pinnagoda, J. & Tupker, R.A. (1995). Measurement of the Transepidermal Water Loss. In *Methods in skin research* pp. 173-178. CRC Press.

Ramachandran, S., Hoban, P.R., Ichii-Jones, F. & et al. (2000). Glutathione S-transferase GSTP1 and cyclin D1 genotypes: association with numbers of basal cell carcinomas in a patient subgroup at high-risk of multiple tumours. *Pharmacogenetics*, **10(6),** 545-546.

Reed, R.J. (1990). Actinic keratoacanthoma; Speculations on the nature of the lesion and the role of cellular immunity in its evolution. *Am J Dermatopathol*, **12(5),** 517-533.

Rees, J.L., Friedman, P.S. & Matthews, J.N.S. (1990). The influence of area of application on sensitisation by dinitrochlorobenzene. *Br J Dermatol*, **122(1),** 29-31.

Rheins, I.A. & Nordlund, J.J. (1986). Modulation of the population density of identifiable epidermal Langerhans cells associated with enhancement or suppression of cutaneous immune reactivity. *J Immunol*, **136,** 867.

Robinson, T.A. & Kligman, A.M. (1975). Treatment of solar keratoses of the extremities with retinoic acid and 5-fluorouracil. *Br J Dermatol* , **92,** 703.

Rook, A.H., Jaworsky, C., Nguyen, Y. & al, e. (1995). Beneficial effect of low-dose systemic retinoid in combination with topical tretinoin for the treatment and prophylaxis of premalignant and malignant skin lesions in renal transplant recipients. *Transplantation* , **59(5),** 714-9.

Rosenberg, S.A., Aebersold, P., Cornetta, K. & al, e. (1990). Gene transfer into humans - immunotherapy of melanoma using tumour-infiltrating lymphocytes modified by retroviral gene transduction. *NEJM*, **323,** 570-8.

Sarkany, I. (1972). Malignant melanomas in lymphoedematous arm following radical mastectomy for breast carcinoma. *J R Soc Med*, **65,** 253-4.

Saunder, D.W., Tamaki, K., Moshell, A.N., Fujiwara, H. & al, e. (1981). Induction of tolerance to topically applied TNP-conjugated UV irradiated epidermal cells. *J Immunol*, **127,** 261.

Schaafsma, M.R., Stijnen, T., Van Es, L.A. & Paul, L.C. (1989). Lack of correlation between the DNCB skin test reactivity and renal transplan survival. *Clin Transplant*, **3,** 19-21.

Scheffner, M., Werness, B.A., Huibregste, J.M. & et al. (1990). The E6 oncoprotein encoded by human papilloma virus types 16 and 18 promotes the degradation of p53. *Cell*, **63,** 1129-1136.

Schreiner, T., Broska, J. & Fierlbeck, G. (1995). Topical application of tretinoin, interferon beta and their combination in the treatment of warts. *J Derm Treatm* , **6,** 17-9.

Serrano, M., Hannon, G.J. & Beach, D. (1993). A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature*, **366,** 704-707.

Servitje, O., Seron, D., Ferrer, I. & et al. (1991). Quantitative and morphometric analysis of Langerhans cells in non-exposed skin in renal transplant patients. *J Cutan Pathol*, **18(2),** 106-111.

Setlow, R.B. & Woodhead, A.D. (1994). Temporal changes in the incidence of malignant melanoma: explanation from action spectra. *Mutation Research*, **307,** 365-74.

Shah, K.V. & Howley, P.M. (1996). Papillomaviruses. In *Fields Virology*, Fields, B.N., Knipe, D.M., Howley, P.M. & al (eds) pp. 2077-2109. Lippincott-Raven: Philadelphia.

Sheil, A.G.R. & (Ed), A.P.S.D. (1991). ANZDATA 14th Report. Australia and New Zealand Transplant Registry: Adelaide. South Australia.

Sheil, A.G.R., Disney, A.P., Mathew, T.H. & et al. (1993). De novo malignancy emerges as a major cause of morbidity and late failure in renal transplantation. *Transplant Proc* , **25(1 pt2),** 1383-4.

Sheil, A.G.R., Disney, A.P.S., Mathew, T.G. & et al. (1992). Malignancy following renal transplantation. *Transplantation Proc* , **24(5),** 1946-7.

Shelley, W.B. & Juhlin, L. (1976). Langerhans cells form a reticuloepithelial trap for external contact allergens. *Nature*, **261,** 46.

Shelley, W.B. & Juhlin, L. (1977). Selective uptake of contact allergens by the Langerhans cell. *Arch Dermatol*, **1139(2),** 187-92.

Shelley, W.B. & Wood, M.G. (1981). Transformation of the common wart into squamous cell carcinoma in a patient with primary lymphoedema. *Cancer*, **48,** 820-4.

Shuttleworth, D., Marks, R., Griffin, P.J.A. & Salaman, J.R. (1988). Treatment of cutaneous neoplasia with etretinate in renal transplant recipients. *Q J Med* , **257,** 717-724.

Sibley, R.K., Dehner, L.P. & Rosai, J. (1985). Primary neuroendocrine (Merkel cell?) carcinoma of the skin. I. A clinicopathologic and ultrastructural study of 43 cases. *Am J Surg Pathol* , **9,** 95-108.

Sontheimer, R.D., Bergstresser, P.R., Gailiunas, P. & et al. (1984). Perturbation of Langerhans cells in immunosuppressed human renal allograft recipients. *Transplantation*, **37(2),** 168-74.

Stark, L.A., Arends, M.J., McLaren, K.M. & et al. (1994). Accumulation of p53 is associated with tumour progression in cutaneous lesions of renal allograft recipients. *British Journal of Cancer*, **70(4),** 662-667.

Steiner, G., Wolff, K., Pehamberger, H. & Stingl, G. (1985). Epidermal cells as accessory cells in the generation of allo-reactive and hapten specific cytotoxic T lymphocytes (CTL) responses. *J Immunol*, **134(2),** 736-41.

Stewart, F.W. & Treves, N. (1948). Lymphangiosarcoma in postmastectomy lymphoedema. *Cancer*, **1,** 64-81.

Stingl, G., Gazze-Stingl, L.A., Abere, W. & et al. (1981). Antigen presentation by murine epidermal Langerhans cells and its alterations by ultraviolet B light. *J Immunol*, **127(4),** 1707-13.

Stingl, G., Katz, S.I., Clement, L. & et al. (1978). Immunological functions of Ia-bearing epidermal Langerhans cells. *J Immunol*, **121(5),** 2005-13.

Storey, A., Thomas, M., Kalita, A. & et al. (1998). Role of a p53 polymorphism in the development of human papilloma-virus-associated cancer. *Nature*, **393,** 229-234.

Sunderkotter, C., Kalden, H. & Luger, T.A. (1997). Aging and the skin immune system. *Arch Dermatol*, **133,** 1256-62.

Sunderkotter, C., Kalden, H. & Luger, T.A. (1997). Aging and the skin immune system. *Arch Dermatol*, **133,** 1256-62.

Surentheran, T., Harwood, C.A., Spink, P.J. & et al. (1998). Detection of HPV DNA in non-melanoma skin cancer from immunocompetent and immunosuppressed individuals. In *Human Papillomaviruses in Dermatology. Satellite Symposium of the International Investigative Dermatology Meeting* pp. 7: Vaals, Netherlands.

Tagami, H. (1995). Measurement of electrical conductance and impedance. In *Methods in skin research* pp. 159-164. CRC Press.

Tam, M. & Cooper, A. (1987). The use of isotretinoin in a renal transplant with acne. *Br J Dermatol* , **116,** 463.

Tatnall, F.M. & Mann, B.S. (1985). Non-Hodgkin's lymphoma of the skin associated with chronic limb lymphoedema. *Br J Dermatol*, **113,** 751-6.

Tefany, F.E., Barnetson, R.S., Halliday, G.M. & et al. (1991). Immunohistochemical analysis of the cellular infiltrate in primary regressing and non-regressing malignant melanoma. *J Invest Dermatol*, **97(2),** 197-202.

Tennant, R., Wigley, C. & Balmain, A. (1999). Chemical carcinogenesis. In *Cellular and molecular biology of cancer*, Franks, L.M. & Teich, N.M. (eds). Oxford University Press.

Therapeutic Drugs. Churchill Livingstone.

Thiel, G., Bock, A., Spondlin, M. & al, e. (1994). Long-term benefits and risks of cyclosporine A (Sandimmune)-an analysis at 10 years. *Transplant Proc*, **26(5),** 2493-8.

Thivolet, J. & Nicolas, J.F. (1990). Skin ageing and immune competence. *Br J Dermatol*, **122 Suppl 35,** 77-81.

Touzeau, O., Borderie, V.M. & Lacroche, L. (1999). Carcinoma of the corneoscleral limbus in a patient treated with cyclosporine after heart transplantation. *N E J M* , **341**, 374-5.

Urabe, A., Haftek, M., Kanitakis, J. & et al. (1989). Cyclosporine A does not modify Langerhans cell number and distribution in normal human skin. *Acta Dermatol Venereol (Stockh)*, **69(3),** 249-52.

Vandeghinste, N., Bersaque, J.D., Geerts, M.L. & Kint, A. (1992). Acitretin as cancer chemoprophylaxis in a renal transplant recipient. *Dermatology* , 185, 307-8.

Vonesh, E.F. & Cinchilli, V.M. (1997). *Linear and nonlinear models for the analysis of repeated measurements*. Marcel Dekker Inc.: New York.

Walder, B., Robertson, M. & Jeremy, D. (1971). Skin cancer and immunosuppression. *Lancet* , **2**, 1282.

Walker, R.G. & d'Apice, A.J.F. (1988). Nonspecific immunosuppression: azathioprine and steroids. In *Renal transplantation: principles and practice*, Morris, P.J. (ed) pp. 202-32. W B Saunders.

Waxman, M., Fatteh, S., Elias, J.M. & et al. (1984). Malignant lymphoma of the skin associated with postmastectomy lymphoedema. *Arch Path Lab Med*, **108,** 206-8.

Weedon, D. (1997a). Lentigines, naevi and melanomas. In *Skin pathology* pp. 673-712. Churchill Livingstone.

Weedon, D. (1997b). Tumours of cutaneous appendages. In *Skin pathology* pp. 713-758. Churchill Livingstone.

Weedon, D. (1997c). Tumours of the epidermis. In *Skin pathology* pp. 635-72. Churchill Livingstone.

Weinberg, R.A. (1986). Oncogenes, antioncogenes and the molecular bases of multistep carcinogenesis. *Cancer Res*, **49,** 3713-21.

Welsh, K.I. & Bunce, M. (1999). Molecular typing for the MHC with PCR-SSP. *Immunogenetics Reviews*, **1**, 156-176.

Westphal, E., Leimenstoll, G., Derger, P. & al, e. (1986). Correlation between reactivity in a DNCB skin test and the survival rate of kidney grafts under cyclosporine. *Transplant Proc*, **18,** 1080-1.

Whittam, L.R., Coleman, R. & MacDonald, D.M. (1996). Plasma cell tumour in a renal transplant recipient. *Clin Exp Dermatol* , **21**, 367-369.

Wilkinson, D.S., Fregert, S., Magnusson, B. & al, e. (1970). Terminology of contact dermatitis. *Acta Derm Venereol*, **50,** 287-92.

Willis, C.M. (1996). The Histopathology of irritant contact dermatitis. In *The irritant contact dermatitis syndrome*, Van der Valk, P.G.M. & Maibach, H.I. (eds) pp. 291-303. CRC Press.

Wilson, A.G., de Vries, N., Pocoit, F. & Duff, G.W. (1993). An allelic polymorphism within the human tumour necrosis factor alpha promoter region is strongly associated with HLA A1, B8 and DR3 alleles. *J Exp Med*, **177**, 557-560.

Winsey, S.L., Haldar, N. A., Marsh, P.H. & al (2000). A variant within the DNA repair gene XRCC3 is associated with the development of melanoma skin cancer. *Cancer Research*, **60**, 5612-16.

Wolfel, T., Klehman, E., Muller, C. & al, e. (1989). Lysis of human melanoma cells by autologous cytolytic T cell clones. Identification of human histocompatibility leukocyte antigen A2 as a restriction element for three different antigens. *J Exp Med*, **170,** 797-810.

Young, A.R. & Walker, S.L. (1995). Photoprotection from UVR-induced immunosuppression. In *Photoimmunology*, Krutmann, J. & Elmets, C.A. (eds), Vol. Cambridge, UK. pp. 285-97. Blackwell Science Ltd, University Press.

Ziegler, A., Jonason, A.S., Leffell, D.J. & et al. (1994). Sunburn and p53 in the onset of skin cancer. *Nature*, **372,** 773-776.

Zimmerman, P., Hunter, J., Bailey, E. & et. al. (1996). Tumour necrosis factor alpha promoter mutation frequencies among four North American racial groups. *Hum Immunol*, **49(S1)**, 52.

Glossary

Apoptosis. Form of cell death in which the cell activates an internal death programme.

Proliferating cells frequently undergo apoptosis, which is a natural process in development and proliferating lymphocytes undergo high rates of apoptosis in development and during immune responses. It contrasts with necrosis, death from without, which occurs in situations such as poisoning and anoxia.

CD4. Molecule on the surface of thymocytes, helper and inflammatory T cells, monocytes and macrophages, which acts as a co-receptor for MHC class II molecules.

CD8. Molecule on the surface of thymocyte subsets and cytotoxic T cells which acts as a co-receptor for MHC class I molecules.

Chemokines. Small cytokines that are involved in the migration and activation of cells, especially phagocytic cells and lymphocytes, which play a central part in the inflammatory response.

Dielectric. Insulated substance offering great resistance to passage of electricity by conduction.

Dyskeratosis. Any alteration in the keratinization of the epithelial cells of the epidermis.

Intercellular adhesions molecules (ICAM) ICAM-1, ICAM-2, ICAM-3. Cell surface molecules that are ligands for the leukocyte integrins and play a role in the binding of lymphocytes and other leucocytes to antigen presenting cells and endothelial cells.

Interferons (IFN) α, β and γ. Cytokines that can induce cells to resist viral replication.

IFN-γ is a product of inflammatory CD4 T cells, CD8 T cells and natural killer cells.

Major histocompatibility complex (MHC). Cluster of genes on human chromosome 6 or mouse chromosome 17 that encodes the MHC molecules involved in antigen

presentation. The MHC also encodes proteins involved in antigen processing and host defence.

Parakeratosis. Persistence of nuclei within the keratinocytes of the stratum corneum or epidermis or mucosal layers, which indicates a partial keratinization process.

Spongiosis. Intercellular oedma of the spongy layer of the skin.

T_H1 cells. Alternative name for inflammatory CD4 T cells

T_H2 cells. Alternative name for helper CD4 cells.

Ultraviolet. Rays emitted by natural and artificial sources with wavelenghts between 290 and 400 nanometers (nm). The ultraviolet spectrum is split into: UVA with wavelengths of 320-400 nm, UVB with wavelengths of 290-320 nm and UVC with wavelengths below 290 nm.