

## **Deciphering the contribution of $\gamma\delta$ T cells to outcomes in transplantation**

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## **Abbreviations**

ACAID, anterior chamber-associated immune deviation

ADCC, antibody-dependent cellular cytotoxicity

alloHSCT, allogeneic haematopoietic stem cell transplantation

$\alpha\beta$ TCR, alpha-beta T cell receptor

BAL, bronchoalveolar lavage

CD, cluster of differentiation

CM, central memory

CMV, cytomegalovirus

DAMPs, damage-associated molecular patterns

DETC, dendritic epidermal T cells

DSA, donor-specific antibodies

EBV, Epstein-Barr virus

eGFR, estimated glomerular filtration rate

EM, effector memory

EPCR, endothelial protein C receptor

FOXP3, forkhead box P3

$\gamma\delta$ TCR, gamma-delta T-cell receptor

GVD, graft-versus-disease effect

GVHD, graft-versus-host disease

HIV, human immunodeficiency virus

HLA-E, human leukocyte antigen E

HMB-PP, (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate

HMGB1, high-mobility group box 1 protein

iEL, intra-epithelial lymphocyte

IL-, interleukin

IFN, interferon

IPP, isopentenyl pyrophosphate pathway

MICA, major histocompatibility complex class I polypeptide-related sequence A

OB, obliterative bronchiolitis

pAg, phosphoantigen

PTX3, pentraxin-related protein

PV, portal vein

RAG, recombination-activating gene

RANTES, regulated on activation, normal T cell expressed and secreted

TEMRA, terminally differentiated effector memory

Th17, IL-17-producing T-helper cells

TLR4, toll-like receptor 4

TRD, T-cell receptor delta locus

TRG, T-cell receptor gamma locus

V(D)J, variable(diversity)joining

VJC, variable-joining-constant

## **Abstract**

$\gamma\delta$  T cells are a subpopulation of lymphocytes expressing heterodimeric T-cell receptors composed of  $\gamma$  and  $\delta$  chains. They are morphologically and functionally heterogeneous, innate yet also adaptive in behaviour, and exhibit diverse activities spanning immunosurveillance, immunomodulation, and direct cytotoxicity. The specific responses of  $\gamma\delta$  T cells to allografts are yet to be fully elucidated with evidence of both detrimental and tolerogenic roles in different settings. Here we present an overview of  $\gamma\delta$  T cell literature, consider ways in which their functional heterogeneity contributes to the outcomes after transplantation, and reflect upon methods to harness their beneficial properties.

$\gamma\delta$  T cells are a highly conserved lymphocyte subpopulation characterised by both innate and adaptive properties. They are physiologically heterogeneous with functions that include immune surveillance for epithelial dysregulation, antigen recognition with characteristic expansion kinetics, and diverse cytokine and chemokine production.<sup>1</sup>  $\gamma\delta$  T cells underpin the initiation and propagation of the immune response through antigen presentation, interactions with dendritic cells, and priming of  $\alpha\beta$  T cells.  $\gamma\delta$  T cells also have a role in the modulation of immune responses through suppressing lymphocyte proliferation, promoting peripheral differentiation of B lymphocytes, and controlling circulating levels of immunoglobulin.<sup>2-4</sup>

A major limitation to transplantation is the requirement for immunosuppression to control the immune responses that culminate in allograft rejection.<sup>5</sup>  $\gamma\delta$  T cells are well positioned to contribute to the allograft rejection response particularly as a bridge between innate and adaptive immunity. Here we present current perspectives on  $\gamma\delta$  T cell development, classification, and physiology and systematically review current literature regarding their function in transplantation.

### **$\gamma\delta$ T cell development**

In contrast to the ubiquitous  $\alpha\beta$  T cells,  $\gamma\delta$  T cells are typified by a heterodimeric T cell receptor (TCR) consisting of transmembrane  $\gamma$  and  $\delta$  chains.  $\gamma\delta$  T cells comprise between 0.5%-6% of total circulating lymphocytes, 4-10% of circulating CD3<sup>+</sup> cells, and approximately 10-50% of tissue-resident T cell populations.<sup>6-8</sup> Typically,  $\gamma\delta$  T cells are double negative (CD3<sup>+</sup>CD4<sup>-</sup>CD8<sup>-</sup>), although CD4<sup>+</sup>, CD8 $\alpha\alpha$ <sup>+</sup>, CD8 $\beta$ <sup>+</sup> and CD4<sup>+</sup>CD8<sup>+</sup> populations have been described.<sup>9-11</sup> The 150kb T-cell receptor  $\gamma$  (TRG) locus encoding  $\gamma$  variable-joining-constant

(VJC) regions is found on chromosome 7p15 whilst the  $\delta$ -encoding T-cell receptor (TRD) locus is found on chromosome 14q11.2 between V and J segments of the  $\alpha$ TCR locus.<sup>12-15</sup> TRG sequences are broadly common between individuals whilst TRD are unique to each person.<sup>7</sup> TCR formation is contingent upon V(D)J rearrangement. Despite restricted V segments within TRD and TRG loci,  $\gamma\delta$  TCRs have significant theoretical junctional diversity with a potential  $10^{18}$  junctional recombinations compared with  $10^{15}$  for  $\alpha\beta$  TCR and  $10^{11}$  for immunoglobulins.<sup>12,16</sup> However, this theoretical diversity is not realised:  $\gamma\delta$  T cell ontogeny results in distinct subpopulations that arise from the thymus during discrete developmental windows.<sup>17,18</sup>

Both  $\alpha\beta$  and  $\gamma\delta$  T cells share a common progenitor – the double negative (DN) thymocyte ( $CD4^-CD8^-$ ).<sup>19,20</sup>  $\alpha\beta$  lymphocyte precursors transition from DN to double positive (DP) thymocytes ( $CD4^+CD8^+$ ) before ultimately expressing either CD4 or CD8 as single positive thymocytes. At the DN stage, thymocytes display either a pre-TCR or  $\gamma\delta$  TCR complex. Historically this was thought to signify lineage commitment. However, thymocytes expressing the  $\gamma\delta$  TCR complex were subsequently manipulated to differentiate into  $\alpha\beta$  as well as  $\gamma\delta$  T cells.<sup>21</sup> Recently,  $\gamma\delta$  TCR stimulation strength within the thymic microenvironment has been proposed as a model able to account for this observation.<sup>22</sup> Strong  $\gamma\delta$  TCR stimulation of immature DN thymocytes favours  $\gamma\delta$  T cell lineage commitment through the ERK/EGR signalling pathway whilst weak stimulation favours  $\alpha\beta$  TCR development.<sup>6,23</sup>

$\gamma\delta$  T cells have been suggested to play a key role in neonatal immunity during maturation of the  $\alpha\beta$  compartment.<sup>24</sup>  $\gamma\delta$  T cells emerge from the foetal thymus in distinct waves. In mice, an initial wave of  $V\gamma5J\gamma1C\gamma1$   $\gamma\delta$  T cells migrate to the epidermis between day 14 and 18.<sup>6</sup> These dendritic epidermal T cells (DETCs) express a  $V\delta1V\gamma5$  TCR and are so-named due to their characteristic morphology, with apically-anchored dendrites at squamous keratinocyte



junctions and highly mobile basal dendrites.<sup>25</sup> These DETCs are the major population of epidermal T lymphocytes in mice and rats, whilst smaller populations of  $\gamma\delta$  TCR<sup>+</sup> epidermal lymphocytes have been described in humans.<sup>26,27</sup> Tissue-resident  $\gamma\delta$  T cells survey their environment for molecular stress signatures, with key roles demonstrated in negative regulation of cutaneous malignancy and wound healing.<sup>28-31</sup> A second wave of  $\gamma\delta$  T cells migrate to mucosal sites including the reproductive tract, tongue, peritoneal cavity, lung, liver, dermis, and secondary lymphoid organs and are pre-programmed to produce interleukin-17 (IL-17).<sup>16,32</sup> Subsequent waves establish IL-4 and IFN- $\gamma$  producing  $\gamma\delta$  T cell populations in secondary lymphoid tissue, lung, liver and intestinal epithelium.<sup>32</sup> In contrast to these oligoclonal populations, however, studies of human cord blood (CB) have revealed a distinct polyclonal  $\gamma\delta$  T cell population with highly diverse pairings of  $\delta$  and  $\gamma$  gene segments not seen in adults. These CB  $\gamma\delta$  T cells exhibit weak cytotoxic potential and an altered cytokine profile.<sup>33,34</sup>

### **$\gamma\delta$ T cell classification**

In humans,  $\gamma\delta$  T cells can be broadly classified by the TCR  $\delta$  chain into V $\delta$ 2 positive and V $\delta$ 2 negative. Their structural and functional heterogeneity means that  $\gamma\delta$  T cells cannot be regarded as a homogeneous population of cells with a single physiological role.<sup>35</sup>

#### *V $\delta$ 2 positive $\gamma\delta$ T cells*

V $\delta$ 2  $\gamma\delta$  T cells account for >70% of circulating peripheral  $\gamma\delta$  T cells, and the  $\delta$ 2 chain is almost always paired with  $\gamma$ 9. V $\delta$ 2V $\gamma$ 9  $\gamma\delta$  T cells are exclusively found in humans and higher

primates.<sup>36</sup> There are four functional phenotypes—naïve, central memory (CM), effector memory (EM), and terminally differentiated effector memory (TEMRA)—which have individual surface-marker phenotypes, distributions and functions (Table 1).<sup>36</sup>

Vδ2Vγ9 γδ T cells recognise and proliferate following exposure to pyrophosphate-containing phosphoantigens (pAg).<sup>37</sup> Intracellular accumulation of such antigens occurs in dysregulated metabolism of tumour cells, as well as following treatment with aminobisphosphonates such as zoledronic acid. Vδ2Vγ9 γδ T cells lyse paediatric hepatocellular carcinoma cell lines *in vitro* as well as malignant melanoma *in vivo*.<sup>38-40</sup> Aminobisphosphonates have also been successfully utilised to expand peripheral circulating γδ T cells *in vivo* within human cancer immunotherapy trials.<sup>41</sup>

Recognition of pAg also underpins the antimicrobial property of γδ T cells. (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) is a potent Vδ2Vγ9 activator produced by mycobacterium tuberculosis, mycobacterium leprae, plasmodium falciparum, and listeria monocytogenes.<sup>1,42-47</sup>

#### *Vδ2 negative γδ T cells*

Vδ2<sup>neg</sup> γδ T cells are a heterogeneous group with TCRs comprised of Vδ1, δ3, or δ5 and Vγ2, γ3, γ4, γ5, or γ8. Vδ1 γδ T cells predominate in mucosae and at epithelial surfaces where they comprise 10-50% of tissue-resident T cells.<sup>48,49</sup> They function as constant immune surveyors for tissue dysregulation, and following pathogen exposure promote a potent inflammatory

response through production of IL-17 in a TLR4-signalling-dependent manner.<sup>50</sup> A comprehensive characterisation of their ligands remains elusive, but is known to include MHC class I related chain A (via NKG2D), endothelial protein C receptor, HLA-E (via CD93/NKG2A/C receptor), and other HLA Ib proteins such as heat shock proteins.<sup>51,52</sup>

V $\delta$ 2<sup>neg</sup>  $\gamma\delta$  T cells proliferate substantially in response to numerous viruses including CMV, EBV, and HIV.<sup>53-55</sup> This was first demonstrated in renal allograft recipients, with subsequent work ascertaining the generality of this expansion. Exposure to CMV *in utero* produces a V $\delta$ 2<sup>neg</sup> expansion akin to that seen in adults, as does CMV reactivation following alloHSCT.<sup>56,57</sup> Expanded V $\delta$ 2<sup>neg</sup>  $\gamma\delta$  T cells are skewed towards a terminally differentiated effector memory phenotype (Table 2).<sup>58-60</sup> They highly express perforin, granzyme B, and CD158 b/j with lower expression of CD94/NKG2A.<sup>58</sup> V $\delta$ 2<sup>neg</sup>  $\gamma\delta$  T cells have a demonstrable role in the control of CMV disease and limitation of CMV-mediated damage, particularly in susceptible populations such as transplant recipients.

V $\delta$ 2<sup>neg</sup>  $\gamma\delta$  T cells infiltrate various solid tumours and demonstrate anti-tumour, pro-tumour, and immunosuppressive functions.<sup>61</sup> V $\delta$ 1<sup>+</sup>  $\gamma\delta$  T cells lyse malignant melanoma cells and are associated with early tumour stage and the absence of metastatic disease.<sup>39</sup> However, their effects in colon cancer are the opposite, where they are major producers of IL-17 and are associated with disease progression.<sup>62</sup> In the context of breast cancer, infiltrating V $\delta$ 1<sup>+</sup>  $\gamma\delta$  T cells have been observed to suppress naïve and effector T cells.<sup>63</sup>

## **Allograft responses**

Whilst the function of  $\gamma\delta$  T cells is, to a certain extent, ontogenetically determined, they nevertheless retain a degree of functional plasticity dependent upon the activating microenvironment and cytokine milieu. The summary presented below offers a systematic overview by transplanted organ type, highlighting the distinct roles of  $\gamma\delta$  T cells in each tissue.

### *Kidney*

#### 1. Ischaemia reperfusion injury

Ischaemia reperfusion injury is an inevitable result of nephrectomy with microvascular dysfunction, hypoxia, and parenchymal damage triggering a vigorous, classically innate, immune response.<sup>64</sup> The duration of warm ischaemia correlates with long-term graft failure and mortality.<sup>65</sup> Similarly, prolonged cold ischaemia time also increases the risk of graft loss.<sup>66</sup>  $\gamma\delta$  T cells are implicated in the evolution of the initial ischaemic insult through recruitment of classical adaptive immune cells, illustrating their transitional immune functions. Early infiltration of  $\gamma\delta$  T cells following ischaemic insult results in  $\alpha\beta$  T cell infiltration and subsequent renal tubule damage (Figure 1).<sup>67,68</sup>  $\gamma\delta$  T cell knockout mice display a reduced ischaemia reperfusion injury.<sup>69</sup>

#### 2. CMV infection

In transplantation, CMV infection is associated with increased acute rejection rates, graft damage and loss, opportunistic infections, cardiovascular risk, graft artery stenosis, and post-transplantation diabetes.<sup>70</sup> Post-transplantation, expanded CMV-specific  $V\delta 2^{\text{neg}}$  cells

comprise V $\delta$ 1<sup>+</sup>, V $\delta$ 3<sup>+</sup>, and V $\delta$ 5<sup>+</sup> populations that account for the significantly increased numbers of  $\gamma\delta$  T cells.<sup>60</sup> The clinical implications of  $\gamma\delta$  T cell functionality are significant: *late* expansion of V $\delta$ 2<sup>neg</sup>  $\gamma\delta$  T cells is associated with more symptomatic CMV disease, including pyrexia, hepatitis, and leukopenia; higher CMV antigenaemias; and the emergence of mutant CMV strains.<sup>49,71</sup> Patients with primary CMV infection have later V $\delta$ 2<sup>neg</sup>  $\gamma\delta$  T cell expansion as do those with early-onset CMV disease post transplantation. In contrast, patients with late-onset CMV disease have an *earlier* V $\delta$ 2<sup>neg</sup>  $\gamma\delta$  T cell expansion which is associated with a reduced risk of recurrent CMV disease.<sup>72</sup> Despite this, clinical outcomes do not appear to differ between early-onset and late-onset disease, with expanded V $\delta$ 2<sup>neg</sup>  $\gamma\delta$  T cells also able to ameliorate CMV infection independently of expansion kinetics.<sup>71</sup> CMV-expanded V $\delta$ 2<sup>neg</sup> populations have also been observed in heart and lung transplant recipients.<sup>73</sup>

Problematically, the anti-CMV cytotoxic functionality of  $\gamma\delta$  T cells may ultimately result in renal allograft damage through donor-specific antibody (DSA)-mediated antibody-dependent cellular (ADCC) toxicity.<sup>74</sup> DSAs opsonise stromal cells *in vitro* resulting in  $\gamma\delta$  T cell-mediated ADCC whilst *in vivo*  $\gamma\delta$  T cells localise to endothelial cells during episodes of acute antibody-mediated rejection. CD16, a low affinity IgG Fc receptor, is highly expressed by activated  $\gamma\delta$  T cells and is able to promote ADCC in a TCR-independent manner, resulting in degranulation of cytolytic enzymes, high expression of granzyme B and perforin, and IFN- $\gamma$  production.<sup>74,75</sup> The response is potentiated by IL-12 and IFN- $\alpha$ , both of which are produced by monocytes and dendritic cells during CMV infection.<sup>70</sup> For patients with DSA, ADCC mediated by CMV-expanded V $\delta$ 2<sup>neg</sup>  $\gamma\delta$  T cells may be clinically significant, with eGFR inversely correlating to the percentage of CMV-induced  $\gamma\delta$  T cells at 1 year.<sup>74</sup>

### 3. Malignancy

A role for  $\gamma\delta$  T cells in post-transplantation malignancy has also been described. In a longitudinal case-control study of 18 renal transplant recipients, those who subsequently developed malignancies were CMV negative and had significantly lower V $\delta$ 2<sup>neg</sup>  $\gamma\delta$  T cells as a proportion of total lymphocytes. By contrast, a CMV-induced V $\delta$ 2<sup>neg</sup>  $\gamma\delta$  T cell expansion was associated with a decreased risk of malignancy, suggesting a role for  $\gamma\delta$  T cells in immunosurveillance.<sup>76</sup> Furthermore, the action of CMV-expanded  $\gamma\delta$  T cells in response to epidermoid carcinoma or Burkitt lymphoma cell lines appears potentiated, with significantly increased IFN- $\gamma$  production compared to CMV-naïve renal allograft recipients who had subsequently developed cancer.<sup>76,77</sup> Further evidence supporting the protective role of  $\gamma\delta$  T cells is provided by a retrospective cohort study of 13 patients with stage 3 renal cell carcinoma, where there was a positive correlation between peripheral blood numbers of  $\gamma\delta$  T cells and survival.<sup>78</sup> However, others have not found statistically significant increases in the odds of cancer development following renal transplantation in relation to  $\gamma\delta$  T cell immune phenotype.<sup>79</sup>

### 4. Allograft protection

The precise role  $\gamma\delta$  T cells play in renal allograft survival is unclear. For example, there is evidence that  $\gamma\delta$  T cells may be protective. Rats fed oral alloantigen of donor splenocytes have significantly prolonged renal allograft survival associated with increased levels of graft-infiltrating CD8<sup>+</sup>  $\gamma\delta$  regulatory T cells (Tregs) producing IL-10.<sup>80</sup> This protective effect can be transferred between rats via adoptive cell transfer. However, no increased graft survival occurs if the cell transfer is TCR  $\gamma\delta$  depleted.<sup>80</sup> Evidence regarding the activation and

recruitment of  $\gamma\delta$  T cells during rejection episodes is also mixed. Lower frequencies of CD8<sup>+</sup>  $\gamma\delta$  T cells have been observed in patients experiencing acute or chronic rejection compared to healthy controls.<sup>81</sup> In contrast, a significantly increased presence of monocytes, macrophages, and TCR  $\alpha\beta$ <sup>+</sup> or TCR  $\gamma\delta$ <sup>+</sup> lymphocytes has been detected within allografts of patients experiencing acute rejection.<sup>82</sup> Similarly,  $\gamma\delta$  T cells have been found in terminally-rejected transplant nephrectomies although their presence in episodes of acute cellular rejection does not significantly alter long-term graft function.<sup>77</sup>

Hence, while  $\gamma\delta$  T cells may result in allograft damage through promulgating IRI, their role as direct effector cells is not definitive. It is also important to balance these effects in light of their activity in post-transplantation infection and tumour immunosurveillance. No single role can be seen as dominant.

### *Lung and heart*

IL-17 is critical in the pathogenesis of acute lung allograft rejection and obliterative bronchiolitis (OB). IL-17<sup>+</sup>  $\gamma\delta$  T cells, along with IL-17-producing T-helper (T<sub>H</sub>17) cells, are the primary producers of IL-17 within lung allograft tissue and function independently of T<sub>H</sub>17.<sup>83</sup> In a mouse model of lung transplantation, IL-17<sup>+</sup>  $\gamma\delta$  T cell allograft infiltration is significantly increased by post-operative day 21.<sup>84</sup> IL-17<sup>+</sup>  $\gamma\delta$  T cells are rapidly activated, at a rate faster than canonical CD4<sup>+</sup> T<sub>H</sub>17 cells. Both TCR-dependent and TCR-independent pathways have been described, although ligands remain elusive.<sup>50,85-87</sup> IL-17<sup>+</sup>  $\gamma\delta$  T cells are activated by CD4<sup>+</sup> cells and can increase IL-17 production to compensate for T<sub>H</sub>17 deficiency.<sup>83,84,88</sup>

Similarly to lung transplantation, IL-17 production by  $\gamma\delta$  T cells following cardiac transplantation profoundly alters graft outcomes despite initial early evidence suggesting no role for  $\gamma\delta$  T cells in cardiac allograft rejection.<sup>89-92</sup>  $\gamma\delta$  T cells are the major producer of intra-cardiac allograft IL-17 and are implicated in both acute and chronic allograft dysfunction.<sup>93,94</sup> Depletion of  $\gamma\delta$  T cells prolongs graft survival, reduces serum IL-17, reduces inflammatory cell infiltrates, and increases graft infiltration by CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> Tregs.<sup>94-97</sup> In acute allograft rejection,  $\gamma\delta$  T cells expand following exposure to long pentraxin 3 (PTX3), an acute phase protein, with IL-17 production stimulated by IL-23 and IL-1 $\beta$  production from dendritic cells, themselves potentiated by high mobility group box 1 (HMGB1).<sup>98</sup> This HMGB1-TLR4-IL-23-IL-17A axis is also crucial for the pathogenesis of ischaemia-reperfusion injury.<sup>99</sup> The peripheral blood composition of  $\gamma\delta$  T cells following cardiac allograft transplantation is also altered, with an increased proportion of CD25<sup>-</sup>,HLA-DR<sup>-</sup> V $\delta$ 1<sup>+</sup>  $\gamma\delta$  T cells that proliferate in response to IL-2.<sup>100</sup> In mice, knockout of  $\gamma\delta$  T cells abrogates cardiomyocyte apoptosis after transplantation.<sup>101</sup>

$\gamma\delta$  T cells accumulate in the endocardium and myocardium of patients over time, and clonal expansion of V $\gamma$ 3J $\gamma$ 2.1C $\gamma$ 2 transcripts has been found in the coronary arteries of patients who experience chronic rejection.<sup>102,103,104</sup>  $\gamma\delta$  T cells also have a role in the pathogenesis of cardiac allograft vasculopathy. RANTES (regulated on activation, normal T cell expressed and secreted), also known as CCL5, is a chemokine that recruits mononuclear cells to the vascular endothelium and precedes intimal thickening.<sup>105</sup> RANTES is produced by  $\gamma\delta$  T cells which triple in number over the development of mouse allograft vasculopathy, and co-localizes with infiltrating mononuclear cells at sites of intimal thickening in the coronary arteries of human



explanted hearts.<sup>106</sup>  $\gamma\delta$  T cells can also mediate allograft dysfunction via IL-12 and NKG2D pathways: blockade of either pathway results in reduced numbers of IL-17/IFN $\gamma$ -producing  $\gamma\delta$  T cells and ameliorates post-ischaemic cardiomyocyte function.<sup>107,108</sup>

Data from both cardiac and pulmonary transplantation highlight how the previously well-described ability for  $\gamma\delta$  T cells to produce IL-17 ultimately expedites graft rejection via numerous pathways including acute CD4<sup>+</sup> and CD8<sup>+</sup> lymphocyte recruitment and chemokine production orchestrating a chronic inflammatory response. The mechanism by which  $\gamma\delta$  T cells are activated in this context, however, remains unclear.

### *Liver*

The role of  $\gamma\delta$  T cells remains ill-defined in liver transplantation. Liver grafts are unique amongst transplanted organs in that a significant numbers of patients may develop operational tolerance.<sup>109-112</sup>  $\gamma\delta$  TCR lymphocytes are highly represented in the human liver, comprising 24.5% compared with 6% in the peripheral blood (Figure 2).<sup>113</sup> These findings are mirrored in mouse livers where approximately 20% of nonparenchymal cells are  $\gamma\delta$  TCR<sup>+</sup> lymphocytes.<sup>114</sup> Immediately following transplantation there is a substantial reduction in the  $\gamma\delta$  T cell compartment with a subsequent year-long reconstitution.<sup>115</sup> Patients developing spontaneous allograft tolerance have significantly increased CD3<sup>+</sup>  $\gamma\delta$  TCR<sup>+</sup> lymphocytes and a V $\delta$ 1:V $\delta$ 2 ratio of >1.<sup>116</sup> The expanded V $\delta$ 1  $\gamma\delta$  T cell population displays a CD45RA<sup>+</sup>CCR7<sup>-</sup> T-EMRA phenotype and develops independently of the thymus.<sup>60,117</sup> In contrast, healthy controls exhibit a V $\delta$ 2  $\gamma\delta$  T cell predominance (V $\delta$ 1:V $\delta$ 2 of <1).<sup>118,119</sup> V $\delta$ 1:V $\delta$ 2 ratios are highest in immunosuppression-free patients. The emergent circulating V $\delta$ 1  $\gamma\delta$  T cells produce

IL-10, thus promoting a Th2 phenotype.<sup>118</sup> This immune phenotype—characterised by the emergence of Vδ1 γδ T cells into the peripheral circulation—also mirrors that observed during successful pregnancy.<sup>120</sup> The phenotype is reversed during acute allograft rejection episodes where an increased proportion of Vδ2 γδ T cells is associated with more severe transaminitis.<sup>121</sup> This is also paralleled during abortive pregnancies which demonstrate a peripheral Vδ2 predominance.

A putative tolerogenic role of Vδ1 γδ T cells has been supported further by genomic studies, which are able to differentiate tolerant patients from those requiring immunosuppression using gene expression profiling. Upregulation of genes producing the δ chain of γδTCR has been observed in spontaneously tolerant patients. These include the T cell receptor δ, soluble T cell receptor delta chain, and T cell receptor delta diversity 3 loci.<sup>116</sup> In one study, all Vδ1 γδ T cells from patients who spontaneously tolerated hepatic allografts exhibited an identical complementarity determining region 3 (CDR3) that was not found in immunosuppressed patients or those experiencing chronic rejection.<sup>122</sup>

A significantly increased γδ T cell infiltrate producing IL-10 and IL-4 in immunologically-unresponsive rats following pre-transplantation transfusion with donor-specific blood has been observed.<sup>123</sup> This raises the possibility of hepatic-resident γδ T cell populations that could be targeted to induce immune tolerance. However, evidence is far from conclusive. For example, a decreased percentage of γδ T cells to <10% in spontaneously tolerated hepatic allografts and an increased percentage in acutely rejecting livers has been shown.<sup>114</sup> Others have found similar percentages of γδ T cells in patients with stable graft function and those

experiencing varying degrees of cellular rejection.<sup>124</sup> IL-17-producing  $\gamma\delta$  T cells, activated by hepatic stellate cells, have a role in the pathogenesis of liver fibrosis, although there is scarce data at present examining their role in chronic liver transplant rejection.<sup>125</sup>

### *Haematopoietic Stem Cell Transplantation (HSCT)*

HSCT offers potential insights into  $\gamma\delta$  T cell repopulation kinetics, participation in graft-versus-host disease (GVHD), and contribution to the graft-versus-leukaemia (GVL) effect. Recent studies have demonstrated increased disease-free survival and abrogated bacterial infections with no increase in GVHD following enhanced reconstitution of the  $\gamma\delta$  T cell compartment.<sup>126-</sup>  
<sup>129</sup> In mice,  $\gamma\delta$  T cells promote engraftment, enhance haematopoietic reconstitution, and augment donor cell chimerism following allogeneic HSCT.<sup>130</sup>

Following myeloablation and HSCT, the  $\gamma\delta$  T cell compartment is promptly reconstituted and precedes the  $\alpha\beta$  lymphocyte compartment repopulation. Reconstituted  $\gamma\delta$  T cells are fundamentally different to the pre-transplantation  $\gamma\delta$  T cell repertoire and somewhat different to the TRD and TRG repertoire of the donor.<sup>7,40,131</sup> Graft-derived lymphocytes expand following alloHSCT as demonstrated by matched CDR3 regions of TCRV $\delta$ 1 between donor and recipient at 1.5 years post-transplant.<sup>132</sup> Donor T cell engraftment in the thymus and spleen is significantly enhanced by pre- and post-transplant  $\gamma\delta$  T cell infusions.<sup>133</sup> A role for the thymus in regulation of  $\gamma\delta$  T cell expansion is also alluded to by data from an athymic patient treated with allogeneic bone marrow transplantation demonstrating a persistently elevated double-negative peripheral phenotype of which 88% were CD3<sup>+</sup>CD4<sup>-</sup>CD8<sup>-</sup>  $\gamma\delta$  T lymphocytes.<sup>134</sup>

CMV reactivation is a significant concern following HSCT. V $\delta$ 2<sup>neg</sup>  $\gamma\delta$  T cell expansion following CMV reactivation has been identified in patients undergoing HSCT, mirroring that observed following renal transplantation. Expanded V $\delta$ 2<sup>neg</sup>  $\gamma\delta$  T cells have an increased effector or terminally differentiated phenotype with fewer central memory cells. They are able to lyse primary leukaemic blasts (but not other tumour cell lines) *in vitro*.<sup>40,135</sup> Proliferated clones following CMV infection also demonstrate a long CDR3 V $\delta$ 2TCR  $\delta$  sequence, mirroring that observed in tolerant liver transplant recipients.<sup>7,122</sup> V $\delta$ 2<sup>neg</sup>  $\gamma\delta$  T cells also have a role in EBV control. In a cord blood transfusion recipient who received CMV<sup>neg</sup> EBV<sup>pos</sup> cord blood, there was a massive, sustained and non-neoplastic V $\delta$ 1<sup>+</sup>  $\gamma\delta$  T cell expansion from 4 to 47% of circulating CD3<sup>+</sup> lymphocytes.<sup>136</sup> These expanded  $\gamma\delta$  T cells demonstrated anti-EBV cytolytic activity.

Evidence for the role of  $\gamma\delta$  T cells in GVHD is mixed. Bone marrow-derived alloHSCT administration of  $\gamma\delta$  T cells in isolation reduces the risk of GVHD whilst co-administration with  $\alpha\beta$  lymphocytes exacerbates it.<sup>137</sup> Naïve  $\gamma\delta$  T cell frequencies are lower in patients who experience GVHD.<sup>138</sup> A subpopulation of Treg cells that expresses  $\gamma\delta$  TCR (FOXP3<sup>+</sup>  $\gamma\delta$ TCR<sup>+</sup>) has been described, which is decreased in patients experiencing chronic GVHD and associated with reduced levels of TGF $\beta$ 1, IL-2, and increased TNF $\alpha$ .<sup>139</sup> On the other hand, increased graft concentrations of  $\gamma\delta$  TCR<sup>+</sup> lymphocytes are associated with an increased frequency of grade II-IV GVHD.<sup>140</sup> There is less histopathological evidence of GVHD in mice administered an anti- $\gamma\delta$  TCR.<sup>141</sup> However, there is evidence that  $\gamma\delta$ TCR<sup>+</sup> cell infusion causes GVHD in mice expressing nonclassical MHC class Ib gene products, but not in mice without.<sup>142</sup>

Harnessing the ability of  $\gamma\delta$  T cells to identify and lyse malignant cells is of potential benefit in the context of haematological malignancy, and evidence thus far demonstrates the feasibility of this approach. Human V $\delta$ 2V $\gamma$ 9  $\gamma\delta$  T cells expanded *in vitro* with zoledronic acid have potentiated cytotoxicity against primary leukaemic blasts, whilst donor-derived V $\delta$ 1<sup>+</sup>CD4<sup>-</sup>CD8<sup>-</sup>  $\gamma\delta$  T cells are activated and proliferate in response to recipient primary acute lymphoblastic leukaemia (ALL) blasts.<sup>40,143</sup> This functionality of  $\gamma\delta$  T cells may underlie the finding that patients treated for ALL or acute myeloid leukaemia with donor alloHSCT have increased leukaemia-free survival that correlates with the number of  $\gamma\delta$  T cells.<sup>126</sup>

### *Skin*

The role of lymphocyte populations in the rejection of skin grafts is well established, and demonstrated by TCR $\beta$  and TCR $\gamma$  knockout mice that tolerate grafts for longer than 160 days.<sup>144</sup> However, there are mixed data regarding the relative contribution of  $\gamma\delta$  T cells in alloreactive pathways. The picture is further obscured by the intra-species variation of  $\gamma\delta$  T cell subpopulations. DETC are the major epidermal T lymphocyte population in mice and rats, whilst smaller populations of  $\gamma\delta$  TCR<sup>+</sup> epidermal lymphocytes exist in humans.<sup>145-147</sup> These cells contribute to tissue homeostasis through cytokine secretion and survey tissue for markers of damage, demonstrated by an increased rate of skin cancer progression in TCR $\delta$ <sup>-/-</sup> mice.<sup>29</sup> DETCs have been observed to cluster with Langerhans cells as they migrate from the epidermis to secondary lymphoid structures.<sup>148</sup> Whether DETCs function as APCs or contribute towards the functional maturation of migratory LCs is unknown.

V $\gamma$ 4  $\gamma\delta$  T cells migrate from the dermis to the epidermis via a chemokine ligand 20 (CCL20)-chemokine receptor 6 (CCR6) pathway in response to skin graft revascularisation. Here, they contribute to a pro-inflammatory microenvironment through the production of IL-17A and IFN- $\gamma$ , resulting in accelerated graft rejection. The response is potentiated by IL-23 and IL-1 $\beta$ .<sup>149</sup> TCR $\delta^{-/-}$  mice demonstrate impaired CD8<sup>+</sup> lymphocyte priming and dendritic cell migration.<sup>150</sup>  $\gamma\delta$  T cells are sufficient for graft rejection in TCR $\alpha\beta$ -deficient mice upon exposure to the non-classical MHC antigen Qa-1, although in this model no rejection results from major or minor histocompatibility complex-mismatched grafts.<sup>46</sup>

There is also evidence for an immunomodulatory role of  $\gamma\delta$  T cells in skin transplantation. Portal vein (PV) immunisation of the graft recipient with hybridoma cells prior to skin grafting induces oligoclonal  $\gamma\delta$  TCR<sup>+</sup> cell expansion and is associated with improved graft survival, enhanced production of IL-4 and IL-10, and suppression of IL-2 and IFN- $\gamma$  production.<sup>151,152</sup> This effect is abrogated in  $\gamma\delta$  TCR knockout mice and by administration of anti-IL-10 and anti-TGF- $\beta$ .<sup>153,154</sup> Two novel molecules proposed to play a role in both successful pregnancy and allograft tolerance are CD200, expressed on dendritic cells and which augments graft survival, and MD-1, which promotes graft rejection. In a mouse model, the rate of skin graft rejection is decreased by infusion of anti-MD1 whilst anti-CD200 blocks the protection afforded by pre-transplantation PV immunisation.<sup>155</sup> Expansion of  $\gamma\delta$  TCR<sup>+</sup> lymphocytes in lymphoid organs following anti-TCR $\alpha\beta$  administration has also been shown, which is associated with improved graft survival and preserved antibacterial action.<sup>43</sup>

### *Other Transplants*

The required immunosuppression in small bowel transplantation is significant, in part due to considerable intra-epithelial lymphocyte (IEL) populations and current limitations with ex-vivo depletion.<sup>156</sup>  $\gamma\delta$  T cells may comprise up to 50% of IELs. In one study, increased graft and animal survival was observed following PV immunisation of rats with donor dendritic cells cultured *ex vivo* from bone marrow aspirates. The improved survival following small bowel transplantation in these rats was associated with increased  $\gamma\delta$  T cell infiltration into the graft, with this effect abrogated by depletion of  $\gamma\delta$  T cells from the adoptive transfer.<sup>157,158</sup> Again,  $\gamma\delta$  T cells produced a Th2-like cytokine phenotype through production of IL-4, IL-10 and TGF $\beta$ .<sup>159</sup> Administration of the monoclonal antibody anti-CD52 (alemtuzumab) prominently depletes the donor-derived  $\gamma\delta$ TCR<sup>+</sup> IEL compartment in a mouse model of small bowel transplantation.<sup>160</sup>

In a mouse model of islet transplantation, long-term survivors after transplantation were observed to have a significant increase in the proportion of CD3<sup>+</sup> lymphocytes expressing a  $\gamma\delta$  TCR.<sup>161</sup> Anterior chamber-associated immune deviation (ACAID), the process by which delayed-type hypersensitivity is averted following inoculation of the anterior chamber of the eye with allogenic material, prevents immune-mediated optic distortion. This immune privilege results in high corneal graft survival. However, infusion of anti- $\gamma\delta$  TCR Ab causes rejection rates to increase from 20% to 75% through inhibition of ACAID formation.<sup>162</sup> Mechanistically, it is thought that  $\gamma\delta$  T cells act via the effector arm of the immune response and are required for the recruitment of Tregs.

## Conclusion

There is an incomplete understanding of  $\gamma\delta$  T biology in transplantation. As highlighted above,  $\gamma\delta$  T cell behaviour is dictated both by their ontogeny and by the specific environment in which they are activated. It is clear that  $\gamma\delta$  T cells are able to drive inflammatory responses through innate-like recognition of tissue damage and the recruitment of adaptive immune cells. Here, their role appears to be dictated by their production of IL-17 which is known to be relevant for the rejection of some types of transplants but not others. Conversely, through the production of Th2-type cytokines, they are able to dampen immune-mediated inflammatory responses and promote tolerance to transplants. Assigning a single role for  $\gamma\delta$  T cells in transplantation as either beneficial or detrimental is not possible, given their wide-ranging activity in both innate and adaptive settings. Further work is required to untangle these conflicting phenotypes, discover activating ligands, and develop potential targets for therapeutic intervention. It is clear that  $\gamma\delta$  T cells must now be viewed with the importance they deserve in transplantation.



Table 1

V $\delta$ 2 $\gamma\delta$ T cell subpopulations	Cell Surface Expression	Localisation	Function
Naïve	CD45RA <sup>+</sup> CD27 <sup>+</sup>	10-20% of peripheral blood $\gamma\delta$ T cells and a major population within lymph nodes.	Activation by IL-2 and IPP results in the development of a T <sub>CM</sub> phenotype  Unable to secrete IFN- $\gamma$
Central Memory (CM)	CD45RA <sup>-</sup> CD45RO <sup>+</sup> CD27 <sup>+</sup> CCR7 <sup>+</sup> CD62L <sup>+</sup>	25% of V $\gamma$ 9V $\delta$ 2 $\gamma\delta$ T cells exhibit T <sub>CM</sub> phenotype in lymph nodes.  50% of V $\gamma$ 9V $\delta$ 2 $\gamma\delta$ T cells exhibit T <sub>CM</sub> phenotype in the peripheral blood.	Activation by IL-2 and IPP results in the development of a T <sub>EM</sub> phenotype
Effector Memory (EM)	CD45RA <sup>-</sup> CD45RO <sup>+</sup> CD27 <sup>-</sup>	This phenotype is scarce in lymph nodes but numerous in	Secrete IFN- $\gamma$ and TNF- $\alpha$  Activated by IPP and IL-2

	CCR7 <sup>-</sup> CD62L <sup>-</sup>	peripheral blood and at inflammatory sites	Stimulation with IL-15 results in the development of a T <sub>EMRA</sub> phenotype.
Terminally Differentiated Effector Memory T Cells	CD45RA <sup>+</sup> CD27 <sup>-</sup> CCR7 <sup>-</sup> CD62L <sup>-</sup> CCR5 <sup>+</sup> CXCR3 <sup>+</sup> Perforin, granulysin.		Terminally differentiated Little proliferative activity Unresponsive to TCR stimulation. Minimal production of IFN-γ

Table 2

Vδ1 γδ T cell subpopulations	Cell Surface Expression	Localisation	Function
Naïve	CD45RA+ CD27+ CD11a <sup>lo</sup>	This phenotype constitutes 80% of cord blood Vδ1 <sup>+</sup> cells  Constant levels of T <sub>naïve</sub> Vδ1 γδ T cells are observed in peripheral blood until late middle age	IL-2 secretion
Non-naïve	CD45RA+ CD27- CD11a <sup>hi</sup>		IFN-γ secretion

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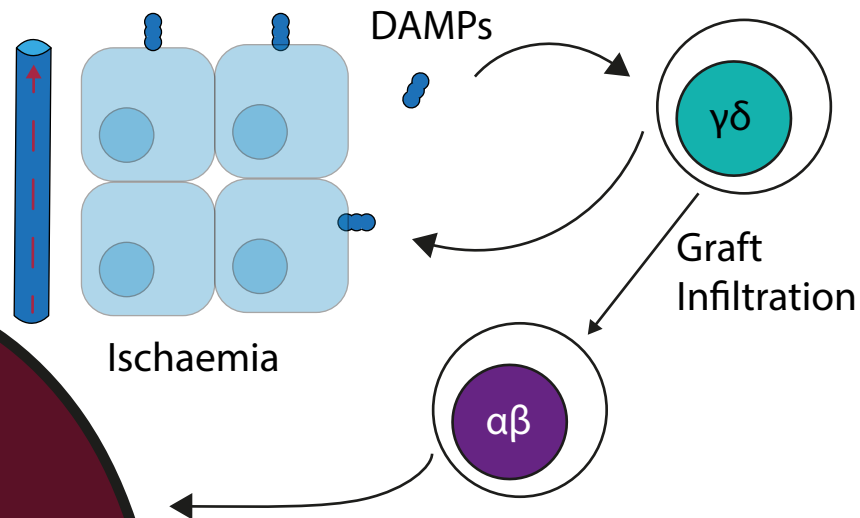


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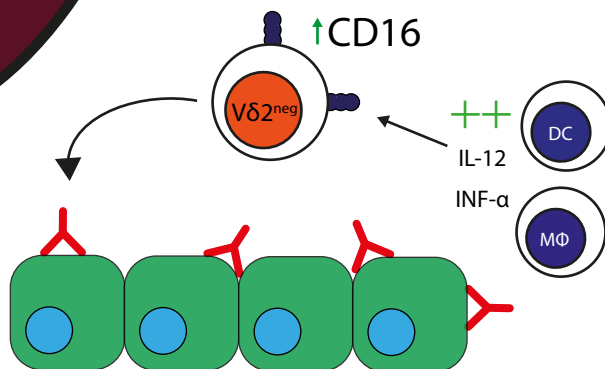
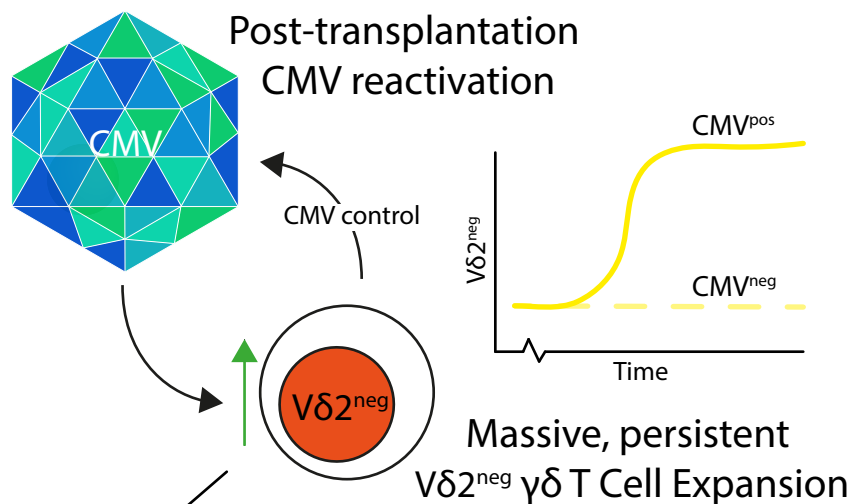
**Figure 1.** A schematic overview detailing the response of  $\gamma\delta$  T cells to renal allografts: (a)  $\gamma\delta$  T cells are activated by ischaemia-induced DAMPs (damage-associated molecular patterns) from the explant resulting in the recruitment of  $\alpha\beta$  T cells; (b) post-transplant reactivation of cytomegalovirus results in a massive, sustained expansion of  $V\delta 2^{\text{neg}}$   $\gamma\delta$  T cells resulting in CMV control; (c) expanded anti-CMV  $V\delta 2^{\text{neg}}$   $\gamma\delta$  T cells might mediate antibody-dependent cellular cytotoxicity against donor-specific antibody opsonised renal endothelial cells and fibroblasts.

**Figure 2.** A schematic overview detailing the response of  $\gamma\delta$  T cells to liver allografts.  $\gamma\delta$  T cells comprise 24.5% of intrahepatic lymphocytes compared to 0.5-6% in the peripheral blood. (a) spontaneous tolerance following liver transplantation is associated with a  $V\delta 1:V\delta 2 > 1$ , and increased production of IL-10 and IL-4. (b) pre-transplantation immunisation in rodent models with donor-derived sensitising antigen (see text) results in improved skin, liver, small bowel, and renal graft survival. Increased IL-10 and IL-4, and decreased IL-2 and IFN- $\gamma$  production have been observed in murine models of skin and liver transplantation. Improved small bowel survival is associated with an increased intra-epithelial  $\gamma\delta$  T cell infiltrate.

(a)  $\gamma\delta$  T Cells recruit  $\alpha\beta$  T Cells in Ischaemia Reperfusion Injury

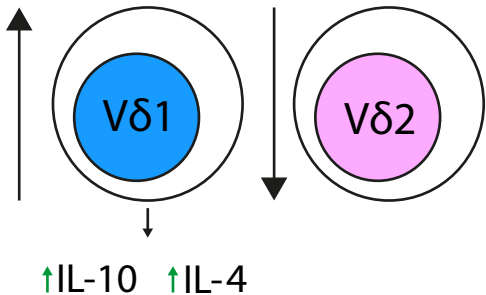


(b)  $\gamma\delta$  T Cells proliferate in response to CMV

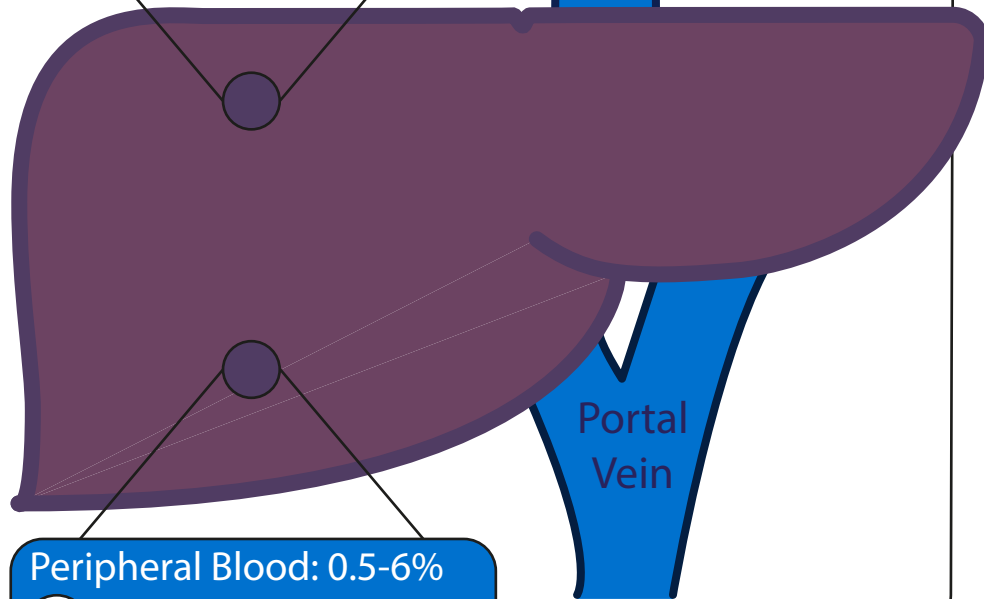
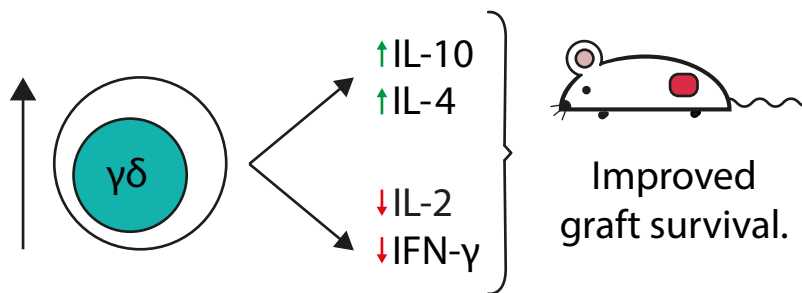


(c) Expanded  $\gamma\delta$  T Cells perform ADCC

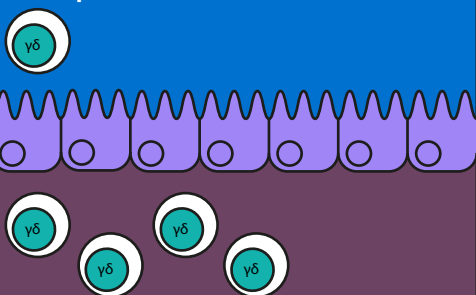
(a) post-liver transplantation immune tolerance phenotype:



(b) Evidence for improved graft survival following pre-transplantation portal vein immunisation:



Peripheral Blood: 0.5-6%



Intrahepatic: 24.5%

Introduction of  
sensitising antigen

