A Polymorphism of Intercellular Adhesion Molecule–1 Is Associated with a Reduced Incidence of Nonmalarial Febrile Illness in Kenyan Children

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An intercellular adhesion molecule–1 polymorphism (ICAM-1^{Kilifi}) is present at a high frequency across sub-Saharan Africa, and its presence may increase susceptibility to cerebral malaria. Here, we report that, compared with children in whom wild-type intercellular adhesion molecule–1 is present, the incidence of nonmalarial fever is significantly lower among those homozygous for ICAM-1^{Kilifi}. We propose that ICAM-1^{Kilifi} may be associated with reduced rates of tissue damage and of death due to sepsis.

Intercellular adhesion molecule–1 (ICAM-1) is a transmembrane glycoprotein expressed on vascular endothelium and leukocytes. ICAM-1 is functionally promiscuous, being both a ligand for molecules (such as leukocyte integrins and fibrinogen) and a receptor for a number of organisms (including *Plasmodium falciparum,* rhinovirus, and coxsackievirus A13). As such, ICAM-1 plays an important role in both the host response to inflammation and the pathogenesis of severe *P. falciparum* malaria [1].

We recently described a polymorphism of ICAM-1 characterized by the substitution of lysine by methionine at codon 29, which we termed ICAM-1^{Kilifi} [2]. Subsequent studies have shown that ICAM-1^{Kilifi} is present at significant and similar frequencies (30%) throughout sub-Saharan Africa [3], is present at a low frequency in Papua New Guinea (5%), and is absent among white and Thai populations [2, 4]. In view of the functional role of ICAM-1 in the sequestration of *P. falciparum* parasites, we hypothesized that, like a number of RBC genetic polymorphisms, the distribution of ICAM-1^{Kilifi} may reflect selection by malaria. However, in a case-control study that was conducted in Kilifi, Kenya, we found that, rather than protecting from severe *P. falciparum* malaria, ICAM-1^{Kilifi} was actually associated with an increased risk of cerebral malaria [2]. Although such an association was not duplicated in a study from The Gambia [3], and although a protective association was found in a study conducted in Gabon [4], ICAM-1^{Kilifi} was associated with an increased risk of death among children with severe malaria in a recent study conducted in Malawi (Gareth Turner, personal communication). Accordingly, how ICAM-1^{Kilifi} has reached its current frequencies remains unknown.

We hypothesized that ICAM-1^{Kilifi} may have been selected through protection against death from diseases other than malaria. Therefore, we studied the genotype-specific incidence of a range of illnesses in children living on the coast of Kenya.

Methods. As described in detail elsewhere [5], subjects enrolled in 2 cohort studies (which were based in the Ngerenya and Chonyi areas of Kilifi District) were monitored by active and passive surveillance for clinical events during the period of September 1998 through August 2001. Children were seen weekly in their homes and, if febrile (axillary temperature, $\geqslant$37.5°C), were referred to a dedicated research outpatient clinic. Children were also free to attend the clinic if they became unwell between visits. At each clinic visit, a clinician obtained a standardized history, conducted an examination, and ascribed a primary diagnosis. ICAM-1 genotype was determined by allele-specific PCR, as described elsewhere [6].

We calculated the genotype-specific incidence of each diagnosis and compared these rates by the calculation of incidence rate ratios using Poisson regression analysis. We adjusted our analysis for the confounding variables hemoglobin type (HbAS or HbAA), age (as a continuous variable), sex, season (defined in 3-month blocks), and ethnic group. Ninety-five percent CIs and significance values were adjusted to take account of potential within-subject clustering of events using the sandwich estimator. Further subanalyses were performed by age group.

Ethics approval for the study was granted by the Kenya Medical Research Institute National Ethical Review Committee. Individual written, informed consent was provided by all study participants or their parents.

Results and discussion. Our study included 455 children who were observed for a total of 40,355 weeks. Thirty-nine participants (8.6%) were homozygous for ICAM-1^{Kilifi}, and 202

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Table 1. The genotype-specific incidence of clinical events.

Visit, diagnosis, ICAM-1 group	All children				Children aged <12 months				Children aged <24 months			
	No. of episodes	Incidence[a]	IRR (95% CI)	*P*	No. of episodes	Incidence[a]	IRR (95% CI)	*P*	No. of episodes	Incidence[a]	IRR (95% CI)	*P*
All clinic visits												
Normal	2611	7.35	1		522	4.58	1		985	6.18	1	
Heterozygote	2392	6.76	0.94 (0.83–1.06)	.324	421	4.68	1.05 (0.85–1.30)	.657	853	5.62	1.02 (0.85–1.23)	.814
Homozygote	404	6.45	0.86 (0.69–1.07)	.170	40	3.23	0.60 (0.44–0.82)	.657	120	4.09	0.77 (0.56–1.06)	.115
Malaria-specific clinic visits: malaria[b]												
Normal	751	2.10	1		60	1.17	1		155	1.50	1	
Heterozygote	666	1.88	0.89 (0.74–1.08)	.242	42	1.08	0.93 (0.56–1.55)	.790	140	1.66	1.02 (0.73–1.43)	.903
Homozygote	135	2.10	0.91 (0.68–1.21)	.518	5	0.79	1.01 (0.36–2.81)	.990	20	1.33	0.80 (0.46–1.39)	.435
Nonmalaria clinic visits												
URTI												
Normal	672	1.89	1		178	1.56	1		316	1.98	1	
Heterozygote	660	1.86	1.01 (0.86–1.19)	.869	164	1.82	1.18 (0.92–1.52)	.203	281	1.85	1.05 (0.84–1.31)	.695
Homozygote	97	1.54	0.81 (0.58–1.13)	.208	13	1.05	0.58 (0.30–1.10)	.097	29	0.99	0.59 (0.35–0.99)	.049
LRTI												
Normal	290	0.82	1		86	0.75	1		162	1.02	1	
Heterozygote	248	0.70	0.91 (0.69–1.20)	.503	69	0.77	1.05 (0.68–1.63)	.827	121	0.80	0.90 (0.63–1.29)	.571
Homozygote	31	0.49	0.61 (0.36–1.01)	.056	11	0.88	0.99 (0.62–1.57)	.960	12	0.55	0.66 (0.43–1.01)	.057
Gastroenteritis												
Normal	227	0.64	1		97	0.85	1		162	1.02	1	
Heterozygote	214	0.60	1.06 (0.82–1.37)	.640	77	0.86	1.04 (0.71–1.53)	.839	146	0.96	1.11 (0.83–1.50)	.474
Homozygote	27	0.43	0.71 (0.50–1.01)	.054	6	0.49	0.51 (0.28–0.94)	.031	14	0.48	0.58 (0.37–0.89)	.014
Skin infection												
Normal	229	0.65	1		51	0.45	1		90	0.56	1	
Heterozygote	265	0.75	1.12 (0.87–1.44)	.376	35	0.39	0.86 (0.47–1.57)	.631	82	0.54	1.02 (0.68–1.54)	.925
Homozygote	59	0.94	1.36 (0.84–2.20)	.215	3	0.24	0.37 (0.10–1.37)	.137	25	0.85	1.50 (0.76–2.94)	.240
Fever of unknown origin												
Normal	128	0.36	1		25	0.22	1		45	0.28	1	
Heterozygote	111	0.31	0.89 (0.67–1.18)	.414	18	0.20	0.92 (0.52–1.64)	.778	35	0.23	0.89 (0.58–1.36)	.590
Homozygote	15	0.24	0.64 (0.38–1.06)	.084	2	0.16	0.67 (0.12–3.72)	.648	7	0.24	0.90 (0.44–1.80)	.757
Helminth infection												
Normal	95	0.27	1		9	0.08	1		20	0.13	1	
Heterozygote	94	0.27	1.00 (0.72–1.38)	.971	4	0.04	0.49 (0.16–1.49)	.210	15	0.10	0.77 (0.38–1.54)	.455
Homozygote	18	0.29	1.07 (0.60–1.92)	.808	1	0.08	1.02 (0.19–5.56)	.974	3	0.10	0.84 (0.26–2.71)	.769
Nonmalarial fever[c]												
Normal	961	2.69	1		309	4.47	1		481	4.66	1	
Heterozygote	911	2.57	1.04 (0.91–1.20)	.542	261	4.53	1.01 (0.82–1.25)	.893	391	4.63	1.02 (0.83–1.25)	.831
Homozygote	131	2.04	0.80 (0.67–0.97)	.022	26	2.63	0.60 (0.44–0.81)	.001	49	3.25	0.76 (0.56–1.03)	.075

NOTE. The overall analysis represents 214 normal subjects with a total of 18,565 child-weeks of follow-up, 202 heterozygous subjects with 18,449 child-weeks of follow-up, and 39 homozygous subjects with 3341 child-weeks of follow-up. Data from children aged <12 months derive from 77 normal subjects (5930 child-weeks of follow-up), 59 subjects who were heterozygous for intercellular adhesion molecule–1 (ICAM-1)^{Kilifi}; 4680 child-weeks of follow-up), and 9 subjects who were homozygous for ICAM-1^{Kilifi} (643 child-weeks of follow-up); and data for children aged <24 months derive from 100 normal subjects (8296 child-weeks of follow-up), 87 subjects who were heterozygous for ICAM-1^{Kilifi} (7890 child-weeks of follow-up), and 17 subjects who were homozygous for ICAM-1^{Kilifi} (1525 child-weeks of follow-up). All analyses were conducted using Stata, version 8.0 (Stata). "Normal" refers to persons homozygous for ICAM-1^{REF}. IRR, incidence rate ratio; LRTI, lower respiratory tract infection; URTI, upper respiratory tract infection.

[a] No. of episodes per child-week of follow-up.

[b] Malaria was diagnosed on the basis of fever (axillary temperature, >37.5°C) with a slide positive for blood-stage asexual *Plasmodium falciparum* parasites at any density. Subjects were considered not to be at risk of malaria and were dropped from both numerator and denominator populations for 21 days after receiving treatment with an antimalaria drug.

[c] Nonmalarial fever was defined as a fever (axillary temperature, >37.5°C) in the presence of a slide negative for *P. falciparum* in a subject who had not received treatment with an antimalarial drug within the preceding 21 days.

(44.5%) were heterozygous for ICAM-1^{Kilifi} (allele frequency, 0.31). In keeping with the results of previous studies [2], we found no evidence of an association between ICAM-1^{Kilifi} and protection from clinical *P. falciparum* malaria (table 1); however, we found that the incidence of episodes of nonmalarial febrile illness was significantly lower among persons who were homozygous for ICAM-1^{Kilifi} (incidence rate ratio [IRR], 0.80; 95% CI, 0.67–0.97; $P = .022$). This effect was greatest during the first year of life (IRR, 0.60; 95% CI, 0.44–0.81; $P = .001$). We found no significant association between the presence of ICAM-1^{Kilifi} and any primary clinical diagnosis in our overall analysis. However, there was a trend toward a reduced incidence of both gastroenteritis and lower respiratory tract diagnoses for persons who were homozygous for ICAM-1^{Kilifi}. These associations were significant in the subgroup of children aged <2 years (IRR for gastroenteritis, 0.58 [95% CI, 0.37–0.89; $P = .014$]; IRR for upper respiratory tract infection, 0.59 [95% CI, 0.35–0.99; $P = .049$]).

Although ICAM-1^{Kilifi} has previously been associated with cerebral malaria [2], we did not expect to find an association with mild clinical disease; the mutation leads to changes in in vitro ICAM-1–parasite binding [7] that may influence the progression from mild to severe disease. However, among children with ICAM-1^{Kilifi} present, we did find a reduced incidence of a range of childhood febrile illnesses that we could not ascribed to malaria. Although our study was not supported by microbiologic evidence, on the basis of our clinical data, it seems unlikely that our findings could be explained by protection from any organism individually, but rather that ICAM-1^{Kilifi} is of integral importance in a variety of different febrile processes. This conclusion raises a number of questions. What is ICAM-1^{Kilifi} protecting against? By what mechanism? And why is its distribution largely restricted to sub-Saharan Africa?

Leukocyte trafficking is an important part of immune defense but also contributes to the pathology of severe sepsis. ICAM-1 is implicated in this process in 2 ways. Leukocytes that are rolling along vascular endothelium become firmly tethered when $\beta 2$ intergin receptors bind ICAM-1. This begins an active process, involving many ICAM-1–dependent intracellular signaling processes, resulting in leukocyte trafficking [8]. In addition, fibrinogen may bind ICAM-1 and integrins to bridge leukocytes and endothelium, enhancing adhesion and trafficking [9]. The reduction of integrin binding to ICAM-1 using antibodies attenuates leukocyte transmigration, markers of tissue injury, and apoptosis in endotoxemic mice [10]. ICAM-1–deficient mice are resistant to the lethal effect of low doses of endotoxin [11].

Functional analyses suggest that, compared with wild-type ICAM-1, the binding of ICAM-1^{Kilifi} to leukocytes is reduced, and binding to fibrinogen does not occur [5], a phenomenon that could result in an overall reduction in leukocyte trafficking across vascular endothelium. We speculate, therefore, that the effect of ICAM-1^{Kilifi} on the incidence of nonmalarial febrile illnesses may reflect its effect on the inflammatory response to infections in general. Even in current era of antibiotics, bacterial sepsis remains a major cause of infant death in sub-Saharan Africa [12]. Therefore, sepsis has exerted a powerful force on the selection of polymorphisms that modulate the inflammatory response. If ICAM-1^{Kilifi} represents such a polymorphism, why is it absent in populations outside of sub-Saharan Africa? The domain containing ICAM-1^{Kilifi} is otherwise highly conserved, suggesting that the vast majority of mutations are deleterious. We therefore speculate that this mutation might have arisen after a rare event early in the history of the African genome, after the departure of European and Asian ancestors. This might explain its consistent presence across the continent and its absence from other populations, many of whom might have benefited from its effect.

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