Roberts, CH; Molina, S; Makalo, P; Joof, H; Harding-Esch, EM; Burr, SE; Mabey, DC; Bailey, RL; Burton, MJ; Holland, MJ (2014) Conjunctival Scarring in Trachoma Is Associated with the HLA-C Ligand of KIR and Is Exacerbated by Heterozygosity at KIR2DL2/KIR2DL3. PLoS neglected tropical diseases, 8 (3). e2744. ISSN 1935-2727

Downloaded from: http://researchonline.lshtm.ac.uk/1621387/

Usage Guidelines

Please refer to usage guidelines at http://researchonline.lshtm.ac.uk/policies.html or alternatively contact researchonline@lshtm.ac.uk.

Available under license: Creative Commons Attribution http://creativecommons.org/licenses/by/2.5/
Abstract

Background: Chlamydia trachomatis is globally the predominant infectious cause of blindness and one of the most common infectious causes of sexually transmitted infection. Infections of the conjunctiva cause the blinding disease trachoma, an immunological pathology that is characterised by chronic conjunctival inflammation and fibrosis. The polymorphic Killer-cell Immunoglobulin-like Receptors (KIR) are found on Natural Killer cells and have co-evolved with the Human Leucocyte Antigen (HLA) class I system. Certain genetic constellations of KIR and HLA class I polymorphisms are associated with a number of disease conditions in which modulation of the innate responses to viral and intracellular bacterial pathogens is central.

Methodology: A sample of 134 Gambian pedigrees selected to contain at least one individual with conjunctival scarring in the F1 generation was used. Individuals (n = 830) were genotyped for HLA class I and KIR gene families. Family Based Association Tests and Case Pseudo-control tests were used to extend tests for transmission disequilibrium to take full advantage of the family design, genetic model and phenotype.

Principle findings: We found that the odds of trachomatous scarring increased with the number of genome copies of HLA-C2 (C1/C2 OR = 2.29 \(p\)-value = 0.006; C2/C2 OR = 3.97 \(p\)-value = 0.0004) and further increased when both KIR2DL2 and KIR2DL3 (C2/C2 OR = 5.95 \(p\)-value = 0.006) were present.

Conclusions: To explain the observations in the context of chlamydial infection and trachoma we propose a two-stage model of response and disease that balances the cytotactic response of KIR expressing NK cells with the ability to secrete interferon gamma, a combination that may cause pathology. The data presented indicate that HLA-C genotypes are important determinants of conjunctival scarring in trachoma and that KIR2DL2/KIR2DL3 heterozygosity further increases risk of conjunctival scarring in individuals carrying HLA-C2.

Introduction

Chlamydia trachomatis (Ct) is an obligate intracellular bacterium [1] which causes significant morbidity as the causative factor of around 106 million new sexually transmitted infections per annum [2]. As the cause of trachoma, the same bacterium is the most common infectious cause of blindness [3]. Ct serovars exhibit highly specific tissue tropism, with serovars A–C being limited to the mucosal epithelium of the ocular conjunctiva. The remaining serovars are sexually transmitted, but whilst serovars D–K are limited to the mucosal epithelium of the genitourinary tract and rectum, the strains L1–L3 are able to invade other tissues including the lymph nodes. Ocular infection in trachoma is spread among young persons through exposure to secretions from the infected eye via direct physical contact, on fomites or by eye-seeking flies [4]. Repeated and prolonged cycles of infection and inflammation have been identified as the main factors that lead to the progressive formation of fibrotic scars on the tarsal conjunctiva, which ultimately becomes deformed. This can cause entropion and trachomatous trichiasis (TT), a condition where the eyelashes turn inwards and irreversibly damage the cornea by scratching the globe of the eye. If left unchecked, TT causes corneal opacity, visual impairment and blindness.

Active trachoma is frequently found in the absence of detectable Ct infection and both tissue damage and scarring are thought to be the result of a chronic immunological reaction [5]. Human conjunctival transcriptome studies in trachoma suggest that in addition to T cell and innate responses of epithelial cells, the
**Author Summary**

*Chlamydia trachomatis* is a pathogen that causes sexually transmitted infections (STIs) and the blinding disease trachoma. Natural Killer (NK) cells are part of the host immune system’s first line of defence against infection. NK cell functions are genetically encoded and differences between individuals mean that some people are better able to respond to infections than others. We found that in certain combinations, specific variants of the gene HLA-C (Human Leucocyte Antigen, C) and of a complex set of genes called the killer-cell Immunoglobulin-like Receptors (KIR) were associated with a six-fold increase in the relative risk of scarring tissue damage resulting from ocular *C. trachomatis* infection (trachoma). This combination of genetic variants may reduce the host’s ability to effectively resolve infections and result in a harmful immune response that ultimately leads to tissue damage and scarring. KIR NK cells are potential cellular mediators of the damaging immune response. Previous studies have identified that the same HLA-KIR genetic constellation that associates with trachoma is actually protective against infectious diseases such as malaria and tuberculosis. The high frequency of the trachoma-associated constellation in African populations may therefore be explained by the evolutionary benefits of protection from the complications of severe disease.

activation and cytotoxic responses of natural killer (NK) cells is an important determinant of the severity of active trachoma [6,7]. NK cells are a rich source of multiple chemokines and cytokines, including interferon gamma (IFNγ), a cytokine that is central to the control of chlamydial intracellular development and growth. IFNγ also has anti-fibrotic properties that can counteract the effects of TGF-β and inhibit fibroblast proliferation and collagen synthesis [8], but when inappropriately expressed may cause immunopathology. NK cells in mucosal-associated lymphoid tissues are known to be important in the maintenance of epithelial cell integrity via production of the cytokine IL-22 [9]. NK cells therefore have the potential to fulfil multiple roles that encompass tissue homeostasis, tissue re-modelling and immunity.

Early studies in murine chlamydial model infections found that NK cell depletion exacerbated disease, delayed clearance and limited the development of specific T cell responses [10,11]. Subsequent studies have confirmed that in response to chlamydial stimulation, NK cells are promoters of T cell immunity and a major source of IFNγ [10,12] but their role as lytic effector cells is less clear. Although *Ct* infected cell lines are lysed *in vitro*, NK cells purified from the peripheral blood of individuals with current chlamydial infection had diminished lytic activity (and reduced IFNγ) compared with uninfected controls [13]. Population diversity in the highly polymorphic genes that encode the variable NK receptors and their ligands [14] along with functional heterogeneity in the NK cell repertoire may account for these findings [15].

Trachoma is a complex inflammatory fibrotic disease in which host polymorphism in immune response genes plays a significant role [16–18]. The conjunctival epithelial surface is compromised in trachoma [5] as a result of the host response to the causative bacterium, which occupies an intracellular niche. Therefore the mechanisms used by NK cells in the control of other intracellular infections such as Hepatitis B [19], Hepatitis C [20] and HIV [21–23] might also be effective against intracellular *Ct*.

NK cells become activated when they are released from inhibition that is normally bound by interaction of specific HLA class I ligands with inhibitory Killer-cell Immunoglobulin-like Receptors (KIRs) [24]. The ligands of several inhibitory KIR have been described including HLA-A3 and HLA-A11 alleles, which are ligands of the KIR3DL2 receptor [25,26] and the HLA-Bw4 public epitope which is the ligand of KIR3DL1 [27,28]. *Homo sapiens* C alleles can be classified (according to a functional dimorphism at amino acid position 80) as carrying one of two KIR binding epitopes, which are known as HLA-C1 and HLA-C2 [29]. The HLA-C2 group of alleles (HLA-C*02/04/05/06…) are ligands of the inhibitory receptor KIR2DL1 [30–32] and its activating counterpart KIR2DS1 [33]. The HLA-C1 group alleles (HLA-Cw*01/03/07/08…) are ligands of both KIR2DL2 and KIR2DL3 [30–32], however, the latter KIR are both able to cross-react (with differing avidities) with a small number of HLA-C2 and HLA-B allotypes [34]. Although germ-line encoded, the KIR gene system is highly polymorphic and exhibits extensive diversity both between individuals and between populations [35–38]. KIRs exhibit haplotype diversity such that different individuals possess variable gene contents. Since KIR and HLA are also found on different chromosomes, individuals can possess a KIR for which they have no cognate ligand, or vice versa. The extensive polymorphism in the KIR system culminates in a repertoire of NK cells within an individual that is more or less sensitive to release from inhibition under appropriate physiological conditions [39]. The strength of the signals mediated by interactions between specific HLA and KIR alleles is also highly variable [29,40,41] and this further limits overall NK cell responsiveness [42]. In part the responsiveness might be predicted by the presence of type ‘A’ and type ‘B’ KIR haplotypes. Type A haplotypes carry genes encoding predominantly inhibitory KIRs. B haplotypes contain some or all of the same genes found on A haplotypes, but additionally may carry the inhibitory KIR2DL2 and KIR2DL3 genes and numerous activating KIRs [36]. KIR haplotypes can be separated in to two variable regions, defined by their orientation towards the centromeric (Cen) or telomeric (Tel) regions of the chromosome [43]. The KIR A and B haplotypes are present in all populations studied to date and are thought to be maintained by the balancing selection pressures of infection, immunopathology and healthy reproduction [44–47]. In recent human history, a wide range of infectious diseases may have reduced the balancing effects in African populations, leading to more directional selection and a unique pattern of HLA and KIR diversity in this region [38,47]. We therefore assessed the extent to which host genotypes at the HLA and KIR loci were associated with trachomatous scarring in a trachoma endemic population from The Gambia.

**Methods**

**Ethics statement**

The study was conducted in accordance with the tenets of the Declaration of Helsinki. The Ethics Committee of the Gambian Government/Medical Research Council Unit, and the ethics committee of the London School of Hygiene and Tropical Medicine approved the study [MRC SCC1177]. Individual written informed consent was obtained from all adult participants. Written informed consent was obtained from a parent/guardian on behalf of those subjects aged <18 years who wished to take part in the study. All samples were anonymised.

**Study population, sampling and ascertainment**

We selected a family study design and identified probands at a relatively early age for clinical signs of conjunctival scarring. This maximised statistical power whilst controlling for population stratification through the use of related control samples. The
was performed using a salting out procedure. Room temperature for up to 6 months [48] before DNA extraction. After collection, brushes were returned to their original packaging and stored dry at (Part Number F-440151, SLS, Nottingham, UK). After collection, were collected from buccal mucosae using sterile cyto-brushes meant that we sampled both biological parents of the probands. In most cases this contributed significantly to the probands’ phenotypes. We rather than environmental factors could be expected to have years). This approach maximised the extent to which genetic contributed to the statistical tests of association.

### Table 1. Clinical and Demographic features of the sample.

| Group     | Age in years (Median [min - max]) | Scarring (C) Grade (n [%]) | No FPC Grading |
|-----------|----------------------------------|--------------------------|----------------|
| **F1 Generation** |                                  |                          |                |
| Total (n = 570)   | 08 [0.1–40] | 386 (67.8) | 48 (8.4) | 112 (19.6) | 20 (3.5) | 4 (0.7) |
| Male (n = 296)    | 08 [0.1–28] | 188 (63.6) | 25 (8.4) | 71 (24.0) | 12 (4.0) | 0 (0.0) |
| Female (n = 274)  | 08 [0.1–40] | 198 (72.2) | 23 (8.4) | 41 (15.0) | 8 (2.9)  | 4 (1.5) |
| Probands (n = 134) | 05 [0.3–22] | 12 (9.0)  | 12 (9.0) | 93 (69.4) | 16 (11.9) | 1 (0.7) |
| Siblings (n = 436) | 10 [0.1–40] | 374 (85.7) | 36 (8.3) | 19 (4.4)  | 4 (0.9)  | 3 (0.7) |
| **Pc Generation** |                                  |                          |                |
| Total (n = 260)   | 39 [18–72] | 121 (46.6) | 65 (25.0) | 69 (26.5) | 4 (1.5)  | 1 (0.4) |
| Female (n = 132)  | 34 [18–65] | 77 (58.2)  | 22 (16.7) | 31 (23.5) | 1 (0.8)  | 1 (0.8) |
| Male (n = 128)    | 45 [23–72] | 44 (34.5)  | 43 (33.6) | 38 (29.7) | 3 (2.3)  | 0 (0.0) |
| Families         | Minimum | 1st Quantile | Median | 3rd Quantile | Maximum |
| Number persons F1 | 1 | 3 | 4 | 5 | 11 |

Sample size and power calculation

An average of 4 offspring per family was assumed with a population prevalence of scarring in those <30 years of age in The Gambia of ~2% [49]. The Pedigree Based Association Test (PBAT) v3.6 program [50] was used to calculate the power of the study to detect with 95% confidence (β<0.05) a genetic association with odds ratios 1.5, 2 and 3 when the hypothetical disease allele had a frequency between 0.01 and 0.50. Figure S1 shows the estimated power of this study to detect genetic associations with trachomatous scarring at a range of allele frequencies and effect magnitudes, given the sample size. We had >90% power to detect an effect size greater than an odds ratio (OR) = 3 when the allele frequency was ≥0.05 and similar power to detect an effect size of OR = 2 when the allele frequency was ≥0.19.

### Table 2. Distribution of age and sex in the study population.

| Group     | Minimum | 1st Quantile | Median | 3rd Quantile | Maximum |
|-----------|---------|--------------|--------|--------------|---------|
| Total (n = 260) | 39 [18–72] | 121 (46.6) | 65 (25.0) | 69 (26.5) | 4 (1.5) |
| Female (n = 132) | 34 [18–65] | 77 (58.2) | 22 (16.7) | 31 (23.5) | 1 (0.8) |
| Male (n = 128)  | 45 [23–72] | 44 (34.5) | 43 (33.6) | 38 (29.7) | 3 (2.3) |

### Statistical analysis

**KIR genotyping for the presence or absence of 17 KIR genes** was performed by PCR using the set of sequence specific primers described by Vilches et al. [53]. The genotyping method was validated by participation in the UCLA Immunogenetics Center KIR exchange programme (http://www.hla.ucla.edu/cellDna.htm). Medium resolution HLA-A, -B and -C genotyping was performed using LABtype sequence specific oligonucleotide probes (OneLambda, Canoga Park, CA, USA) on a Luminex platform (Luminexcorp, Austin, TX, USA). Medium resolution HLA typing data generates strings of possible allele combinations. Information from the HLA genotypes of family members was used to reduce the length of the strings of possible allele pairs and to eliminate alleles that were not compatible with Mendelian inheritance within a given pedigree. Strings were further shortened where possible to include only common and well-defined alleles [54]. In order to maximise statistical power, highly sequence similar HLA alleles were combined in to groups (table S1) before FBAT. KIR ligands of HLA (HLA-A*03/11/Bw4, HLA-B-Bw4, HLA-C1/C2) were inferred from the full HLA genotypes of individual specimens rather than the reduced strings. The HLA-C*16:01 allele has not been observed in other West African populations whilst HLA-C*16:01 is very common (data from allelefrequencies.net). HLA types were used to identify cases of parental mis-assignment and inconsistent parent-offspring genotypes. KIR phenotypes (presence/absence) were tested for Mendelian inconsistencies. KIR2DL5, KIR2DS3 and KIR2DS5 were not included in the association tests as they can segregate to both Cen-B and Tel-B regions and confound haplotype assignments.

### Trachoma phenotypes

Trachoma was graded in the field using the WHO simplified grading system by field supervisors certified for trachoma grading with regular performance checks as described by Derrick et al. [52]. Photographs were subsequently reviewed by two ophthalmologists with experience of grading trachoma and a final grade agreed. Subjects were assigned to the ‘scarred’ group if there were any signs of trachomatous scarring, in either eye. Individuals where phenotypes could not be confirmed for reasons of poor quality photography (n = 5) did not contribute to the statistical tests of association.
R. Family based tests of HLA association were carried out using FBAT v.2.0.3 [55] performing a series of bi-allelic tests (i.e. association of an index allele against all other alleles) under an additive genetic model and the null hypothesis of no linkage and no association of any factor of the HLA system with trachomatous scarring. This approach is robust to effects of population structure [55,56] and is applicable to a data set with samples originating in mixed ethnic backgrounds. We tested for associations between scarring and all HLA alleles with a sample frequency greater than 0.05 with an offset value of 0.02 (population prevalence of scarring in persons ≤30 years of age) to allow the unaffected siblings to contribute to the test statistic. All FBAT p-values were adjusted using a conservative Bonferroni correction. Significant associations were tested again using a case/pseudo-control conditional logistic regression (CLR) [57], which generated estimates of odds ratios and associated p-values. To test for independence between the disease-associated alleles, we included all alleles that had a corrected p<0.05 in a multivariate CLR model. To establish whether significant HLA associations were restricted to F1 subjects with specific KIR genotypes we tested the full data set under a genotype model [58,59], using CLR, in different subsets of the F1 data where the population was limited by the KIR genotype. Because of the high linkage disequilibrium between factors of the KIR system, these tests were not considered to be independent and test statistics were corrected using the Benjamini-Hochberg method.

Results

Sample population

We sampled 830 individuals from 134 pedigrees and 146 nuclear families in which scarring trachoma had been identified in the first filial (F1) generation. The self-described ethnic background of the parental (P0) population (n = 260) was approximately 40% Mandinka, 23% Fula, 15% Jola, 15% Wolof, 5% Bambara and 2% other minority ethnic groups. There were 570 persons in the F1 generation, where the gender distribution was 52% (n = 296) male and 48% (n = 274) female. The median number of offspring per pedigree was 4 (range 1–11). Eight families had one missing parent. There were 180 (32%) cases of trachomatous scarring in the F1 generation and of these, 72 (40%) were female and 108 (60%) were male. Three hundred and eighty six (67.8%) F1

| Locus | Allele Name* | Allele frequency (P0) | # informative families (n) | S-E(S) | Var(S) | Z | P | Corrected P |
|-------|--------------|-----------------------|----------------------------|--------|--------|---|---|-------------|
| HLA-A | A*02:01      | 0.067                 | 37                         | -4.66  | 10.767 | -1.42 | 0.155552 | 1           |
|       | A*23:01      | 0.164                 | 67                         | -0.56  | 24.675 | -0.113 | 0.910241 | 1           |
|       | A*26:01      | 0.063                 | 32                         | -2.74  | 8.366  | -0.947 | 0.343476 | 1           |
|       | A*30:02      | 0.065                 | 28                         | -0.54  | 8.752  | -0.183 | 0.855165 | 1           |
|       | A*33:01      | 0.138                 | 62                         | 2.557  | 18.996 | 0.587  | 0.557473 | 1           |
|       | A*68:01      | 0.05                  | 21                         | 2.95   | 7.209  | 1.099  | 0.27191  | 1           |
|       | A*68:02      | 0.052                 | 27                         | 3.18   | 8.352  | 1.1    | 0.271184 | 1           |
| HLA-B | B*07:02      | 0.067                 | 33                         | -2.66  | 10.335 | 0.827  | 0.407994 | 1           |
|       | B*08:01      | 0.069                 | 41                         | -12.18 | 11.786 | -3.548 | 0.000388 | 0.01         |
|       | B*35:01      | 0.133                 | 51                         | 6.77   | 17.467 | 1.62   | 0.105266 | 1           |
|       | B*15:03      | 0.071                 | 35                         | -0.14  | 12.033 | -0.04  | 0.967807 | 1           |
|       | B*53:01      | 0.123                 | 48                         | 9.62   | 16.341 | 2.38   | 0.017324 | 0.49         |
|       | B*58:01      | 0.075                 | 40                         | 2.28   | 12.467 | 0.646  | 0.518451 | 1           |
|       | B*78:01      | 0.056                 | 29                         | -0.53  | 8.268  | -0.184 | 0.853761 | 1           |
| HLA-C | C*02:02      | 0.114                 | 53                         | -0.29  | 16.851 | -0.071 | 0.943679 | 1           |
|       | C*03:04      | 0.083                 | 45                         | -11.888| 13.791 | -3.201 | 0.001369 | 0.04         |
|       | C*04:01      | 0.186                 | 66                         | 11.629 | 23.828 | 2.382  | 0.017205 | 0.49         |
|       | C*06:02      | 0.089                 | 33                         | 5.52   | 10.042 | 1.742  | 0.081515 | 1           |
|       | C*07:01      | 0.114                 | 55                         | -2.403 | 17.979 | -0.567 | 0.570849 | 1           |
|       | C*16:01/02   | 0.12                  | 52                         | -7     | 14.981 | -1.809 | 0.070526 | 1           |
|       | C*17:01      | 0.054                 | 27                         | 2.834  | 7.569  | 1.03   | 0.302871 | 1           |
| HLA-A3/11 | No KIR epitope | 0.772             | 81                         | -2.41  | 31.893 | -0.427 | 0.669564 | 1           |
| Bw4_80I | 0.183             | 69                         | -0.06  | 26.511 | -0.012 | 0.990703 | 1           |
| HLA-Bw4/Bw6 | Bw6          | 0.625             | 108                        | -3.907 | 42.358 | -0.6   | 0.548333 | 1           |
| Bw4_80I | 0.318             | 101                        | 3.867  | 41.641 | 0.599  | 0.549034 | 1           |
| Bw4_80T | 0.056             | 28                         | 0.04   | 10.116 | 0.013  | 0.989966 | 1           |
| HLA-C1C2 | C2             | 0.499             | 99                         | 23.08  | 40.6   | 3.622  | 0.000292 | 0.008        |

*Named alleles may indicate the first allele identifier in a longer string of related alleles, but these have been shortened for ease of reading. Full details can be found in table S1.

doi:10.1371/journal.pntd.0002744.t002
individuals were unaffected and phenotypic status could not be confirmed for 4 (<1%). Table 1 gives a detailed description of the phenotype distribution in the families. Detailed examination of photographs revealed that 12 probands did not have sufficient signs of trachomatous scarring. One proband could not be graded. In all the families where there was no photography confirmed scarred proband, at least one sibling was identified who was under 30 years of age and had signs of scarring. HLA genotyping identified paternal misassignment in 63 F1 individuals (11%) who were reassigned to an unknown father but were otherwise retained for analysis.

HLA and KIR genotypes

Table 2 shows the Family Based Association Test (FBAT) estimates of the HLA allele and KIR epitope frequencies in the sample population. Figure 1 describes the 64 unique KIR genotypes that were observed in the P0 generation. Thirty-eight additional KIR genotypes were revealed by re-assortment of the parental haplotypes in the F1 generation (Figure 2). All observed genotypes were assigned as either the ‘AA’ or ‘Bx’ genotypes (where Bx includes both AB and BB genotypes) for the full KIR region and where possible, for each of the Cen and Tel regions. A number of unusual genotypes were identified in this population, most notably, 10.4% of P0 individuals (n = 27/260) possessed KIR2DL2 but not KIR2DS2.

Linkage disequilibrium

Pairwise linkage disequilibrium data (LD) for the KIR genes were calculated (figure S2). Contrary to data from other studied human populations [58,60,61] and consistent with other findings within Africa [47], we observed reduced LD between KIR genes. We did not identify any pairs of KIR genes that were in perfect LD ($r^2 = 1$; only two of the four possible haplotypes observed), although a number of KIR genes were found to be in complete LD.
only three of the four possible haplotypes observed). The extent of LD was insufficient for high confidence imputation of missing KIR genotypes for use in FBAT [58].

Association tests

Any HLA alleles and KIR epitopes with estimated frequencies above 0.05 were included in the FBAT. Three sets of HLA alleles were significantly associated with trachomatous scarring (Table 2). These were HLA-B*08:01 (Z = 2.34, p = 0.003, corrected p = 0.01), HLA-C*03:04 (Z = 3.20, p = 0.004, corrected p = 0.04) and the KIR epitope HLA-C1/C2 (Z = 3.62, p = 0.0003, corrected p = 0.008). Only HLA-C1/C2 remained significant (HLA-C2, OR = 1.68, p = 0.003, corrected p = 0.01) in a multivariate case/pseudo-control, additive model that included all three factors (Table 3), indicating that the HLA-C1/C2 epitope was the only significant independent factor of the HLA system that was associated with trachomatous scarring. In line with previous study designs and analyses we divided the data into several subsets [58,59]. We identified that in the majority of subsets, as with the unselected sample, the relative risk of scarring increased with the number of genomic copies of the HLA-C2 epitope in an additive manner (Table 4). The association of the HLA-C2 homozygote genotype with trachomatous scarring was restricted to the subsets of offspring who were KIR2DL2+ and KIR2DL3+ (Cen-AB) (OR = 5.95, p = 0.0025, BH corrected p 0.006) and to those who were KIR3DL1+ KIR3DS12 and KIR2DS12 (Tel-AA) (HLA-C2 homozygote OR = 4.89, p = 0.00006, BH corrected p 0.004). Elevated odds ratios were observed in sensitivity analyses (Table 4) in F1 samples where the case definition was restricted to those with moderate or severe (WHO FPC grade C2 or C3) rather than evidence of any (C1, C2 or C3) scarring.

Comparison of Gambian KIR gene frequencies to other human populations

We used Principle Components Analysis (PCA) to compare the KIR gene frequencies observed in the P0 generation of the Gambian trachoma families to those observed in other populations where data was available (allelefrequencies.net database, Figure 3). The proportions of the total variance explained by the first three principle components were 0.42 (σ = 2.05), 0.28 (σ = 1.69) and 0.11 (σ = 1.03). The P0 specimens clustered with other populations of African descent, which could be recognised by the observation of

Figure 2. KIR genotypes and that were uniquely identified in the F1 population.
doi:10.1371/journal.pntd.0002744.g002

Table 3. Significant results of case/pseudo-control CLR analysis of total family data set.

| Allele | Odds Ratio | P Value |
|--------|------------|---------|
| HLA-B*08:01/* | 0.694 | 0.7000 |
| HLA-C*03:04/* | 0.500 | 0.1500 |
| HLA-C EPITOPE C2 | 1.684 | 0.0033 |

Likelihood ratio test = 23.1 on 3 df, p = 0.0000379 n = 580, number of informative events = 152.
doi:10.1371/journal.pntd.0002744.t003
high frequencies of the genes defining the Cen-B (KIR2DS2, KIR2DL2) and Tel-A (KIR3DL1 and KIR2DS4) haplotypes.

**Discussion**

We identified three factors of the HLA system (HLA-C1/C2, HLA-B*08:01 and HLA-C*03:04) that associated with trachomatous scarring. However, the protective associations of HLA-B*08:01 and HLA-C*03:04 observed by univariate analysis were not independent of the HLA-C1/C2 association under a multivariate model. This dependence can be explained by the presence of a common HLA-B*08~C*03 haplotype, which we estimate to have a frequency of around 5.7% in the Gambian trachoma families. In Senegalese Mandinka, this haplotype has an estimated population frequency of 5.5% [62] whilst in African-Americans the frequency is estimated to be as high as 22% [63]. Any assumption about how the presence of Cen-B alleles [38] might indicate reduced cellular inhibition in Gambians should therefore be made with some caution.

**HLA-C2 epitopes potentially impair NK cell responses and facilitate chronic Ct infection**

The chlamydial protease, CPAF has been reported to interfere with the surface presentation of HLA class I molecules [14,64–66], but recently this has been called into question by Chen et al. [67]. Kagebein et al. [68] then demonstrated that Ct infection does not lead to alteration in normal MHC Class-I expression, maturation or surface presentation. This implies that Ct infected cells are unlikely to be targets for missing-self reactions mediated by NK cells which selectively monitor down-regulation or loss of self-type MHC class I on target cells. Instead it is more likely that cytotoxic NK responses in chlamydial infections are controlled by dynamic changes in the expression levels of activating NK receptors. These changes may occur as a result of infection and other environmental triggers [69,70] and might overwhelm the inhibitory effects of the more strictly expression-regulated [69] NK inhibitory pathways.

HLA-C1:KIR2DL3 inhibited NK cells have weaker inhibitory signals than other HLA-C inhibited cells [29] and may have a lower threshold for activation. Khakoo et al. [40] reported that the HLA-C1/C1 KIR2DL3/2DL3 genotype constellation increased probability of clearance of early stage Hepatitis C Virus (HCV) infections. Ahlenstiel et al. [42] provided evidence that HLA-C1 homozygotes might be better able to challenge early infections by showing that the proportion of the total NK cell repertoire that is educated and inhibited by HLA-C is ~50% greater in this group than that in HLA-C2 homozygotes [42]. The same study showed that HLA-C1 inhibited NK cells are better able to mount rapid, intense responses to infection through degranulation and IFNγ secretion [42]. In Ct infections, HLA-C1/C1 individuals may be able to limit chronicity by controlling the early stages of Ct infections with an NK response that is easily activated, and involves a more substantial component of the NK repertoire than in HLA-C2/C2 individuals. This may also be true of HLA-C2 individuals who possess only weakly responsive KIR2DL1 alleles, such as those alleles that are found on the commonest B haplotypes in Caucasian populations [41]. However, in the Ga-Adangbe population of Ghana, there was a great diversity B haplotypes, none of which were found at high frequency and many of which carried non-attenuated KIR2DL1 alleles [38]. Any assumption about how the presence of Cen-B might indicate reduced cellular inhibition in Gambians should therefore be made with some caution.

**A role for within-person inhibitory KIR diversity in influencing immunopathology**

The role of KIR in mediating NK cytotoxic responses is well studied, but it is now clear that KIR expressing NK cells are also a major source of IFNγ [71]. The ability of NK cells to produce IFNγ in response to microbial stimuli is related to the density of NCAM-1 (CD56) expressed on their surface, their KIR genotype and the degree of stimulus by accessory cells. An indication of the strength of regulation imposed by the KIR genotype can be estimated as a ratio, known as the ‘DIM factor’, between the response of the CD56dim

| Table 4. Subset analysis of HLA-C1C2 genotype associations with scarring. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Offspring Genotype | Genotype test HLA-C1/C2 | BH Corrected P | Genotype test HLA-C2/C2 | BH Corrected P | n | Number of events |
|-------------------|---------------------|----------------|---------------------|----------------|---|-----------------|
| Unselected        | OR = 2.29 p = 0.0026 | 0.061          | OR = 3.97 p = 0.00005 | 0.0004         | 636 | 159            |
| KIR2DL2/KIR2DL3    | OR = 1.94 p = 0.08  | 0.120          | OR = 2.00 p = 0.15   | 0.191          | 296 | 74             |
| KIR2DL2/KIR2DL3    | OR = 2.33 p = 0.057 | 0.100          | OR = 5.95 p = 0.0025 | 0.006          | 240 | 60             |
| KIR2DL2/KIR2DL3    | OR = 1.5 p = 0.73   | 0.786          | OR = 6.00 p = 0.13   | 0.182          | 76  | 19             |
| KIR3DS1/KIR3DS1    | OR = 2.86 p = 0.0013| 0.006          | OR = 4.89 p = 0.00006| 0.0004         | 524 | 131            |
| KIR3DS1/KIR3DS1    | OR = 0.52 p = 0.29  | 0.338          | OR = 0.90 p = 0.89   | 0.890          | 88  | 22             |
| Affected cases defined by more severe scarring (WHO FPC score C2 or C3) & KIR2DL2/KIR2DL3 (Cen-AA) | OR = 2.07 p = 0.026 | 0.052          | OR = 3.57 p = 0.0017 | 0.006         | 444 | 111            |

doi:10.1371/journal.pntd.0002744.t004

PLOS Neglected Tropical Diseases | www.plosntds.org 7 March 2014 | Volume 8 | Issue 3 | e2744
(KIR-HLA dependent) and CD56\textsuperscript{bright} (KIR-HLA independent) IFN\gamma responding populations [71]. The majority of human NK cells in the periphery are CD56\textsuperscript{dim}, express KIR and are susceptible to inhibition through KIR-HLA interaction. KIR genotype directly influences the DIM factor, but the exact genotypic conformation that defines this has yet to be elucidated. It has been proposed that the NK cell IFN\gamma response will be higher in individuals with more KIR educated NK cells, a situation found when there is a greater diversity of within-person inhibitory KIR genes. Experimentally, IFN\gamma production in CD56\textsuperscript{dim} NK cells showed least inhibition (and

![Figure 3. Principle Components Analysis of Gambian KIR frequencies and other world populations. African populations are characterised by high frequencies of the Cen-B (KIR2DS2—KIR2DL2, KIR2DL2) and Tel-A (KIR2DS1—KIR2DS4) haplotypes. Gambian samples, including the P\textsubscript{r} specimens and malaria cases cluster together and have some of the highest observed frequencies of Cen-B and Tel-A. The proportions of the total variance explained by the first three principal components were respectively 0.42, 0.28 and 0.11. Footnote to figure 3. NO, TS : Parents from Gambian trachoma families, unaffected, TS : Parents from Gambian trachoma families, affected, GAM, SM : Gambian severe malaria cases, GAM, M : Gambian uncomplicated Malaria cases, GCB : Gambian cord bloods, COM : Comoros, ENG : Equatorial New Guinea, GHA : Ghana, IC : Ivory Coast, MO : Morocco, SEN : Senegal, SA : South Africa, EN6 : England – West Midlands Afro-Caribbean, GUA : Guadeloupe, MAR : Martinique, REU : Reunion, TAF (Trinidad Africans), USAF : USA Californian African Americans.

doi:10.1371/journal.pntd.0002744.g003]
the highest DIM factor) in KIR AB heterozygotes [71]. In HLA-C2 homozygotes, we observed a significant KIR2DL2/L3 heterozygote (Cen-AB) disadvantage (Table 4) and an increased relative risk in those with the Tel-AA genotype. The number of persons with Tel-B genotypes was very low in this study, which reflects the low diversity in the Tel region that was reported in another West African population [38]. The high phenotypic frequency of KIR3DL1 (Tel-A) in this Gambian population (~99.6%) indicates that most individuals with the Cen-AB genotype possess at least one Tel-A haplotype. The Cen-AB, Tel-A' genotype represents a full complement of the known MHC specific inhibitory KIRs (KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL1 and KIR3DL2) and this genotype might define a high DIM factor [71]. NK cell clones with a Cen-AB genotype would therefore be relatively resistant to inhibition (DIM factor >1) and would retain the potential for high IFNγ production.

Common tropical infectious diseases drive selection of high frequencies of trachoma risk genotype constellations

The KIR system exhibits extensive diversity in African populations [47,72,73] possibly driven by a high burden of life threatening infectious diseases, that have exerted strong (diversifying) selective pressures on each population [46,47,74]. The high prevalence of Ct STIs in some African populations has been implicated as a contributory factor to the high incidences of infection related infertility that are observed in Africa [75]. It is therefore surprising that Ct disease associated KIR and HLA genotypes are enriched in Africa. One explanation is that opposing selection pressures from other infectious diseases negate selection by Ct. Our sample was selected based on disease phenotype and we found KIR gene frequencies similar to other African populations (Figure 3). The Gambian samples are clearly separated from those in other geographical regions by high frequencies of the genes that define the Cen-B and Tel-A haplotypes (Figure 3) [72]. The frequency of HLA-C2 epitopes is reported to be higher in African populations than in other populations [46,76,77] and the HLA-C epitope frequencies that we observed are similar to those previously described [77]. Yindom et al. [78] reported that the proportion of persons with the constellation HLA-C1 and KIR2DL2/KIR2DL3 (Cen-B) is higher in cases of malaria than in population matched, cord-blood controls [78]. In a study of a South-East Asian population, Hirayasu et al. [79] reported that natural selection may have
of susceptibility to chlamydial genital infection. Infect Immun 66: 5867–5875.

3. Harding-Esch EM, Edwards T, Mkocha H, Munoz B, Holland MJ, et al. (2010) Trachoma prevalence and associated risk factors in the gambia and Tanzania: rapid elimination of trachoma (PRET) team, MRC laboratories The Gambia and to the families and individuals who consented to participate in this study.

4. Abdelrahman YM, Belland RJ (2005) The chlamydial developmental cycle. FEMS Microbiol Rev 29: 949–959. doi:10.1016/j.femsre.2005.03.002.

5. Hu VH, Holland MJ, Burton MJ (2013) Trachoma: protective and pathogenic ocular immune responses to Chlamydia trachomatis. PLoS Negl Trop Dis 7: e2020. doi:10.1371/journal.pntd.0002020.

6. Hu VH, Weiss HA, Ramadhas AM, Tolbert SB, Massar P, et al. (2012) Invasive immune responses and modified extracellular matrix regulation characterize bacterial infection and cellular/connective tissue changes in scarring trachoma. Infect Immun 80: 121–130. doi:10.1128/IAI.05963-11.

7. Natvidad A, Freeman TC, Jeffries D, Burton MJ, Mabey DCW, et al. (2010) Human conjunctival transcriptome analysis reveals the prominence of innate defense in Chlamydia trachomatis infection. Infect Immun 78: 4895–4911. doi:10.1128/IAI.00844-10.

8. Gujriyalalshri G, Giri SN (1995) Molecular mechanisms of antifibrotic effect of interferon gamma in bleomycin-mouse model of lung fibrosis: downregulation of TGF-beta and procollagen I and III gene expression. Exp Lung Res 21: 791–801. doi:10.1128/IAI.00843-10.

9. Tseng CT, Rank RG (1998) Role of NK cells in early host response to Chlamydia trachomatis infection. Infect Immun 66: 5867–5875.

10. Nagarpuram UM, Sikes J, Pannett D, Andrews GW, Frazer L, et al. (2011) MyD88 deficiency leads to decreased NK cell gamma interferon production and T cell recruitment during Chlamydia muridarum genital tract infection, but a predominant Th1 response and enhanced mononuclear inflammation are associated with infection resolution. Infect Immun 79: 486–498. doi:10.1128/IAI.00843-10.

11. Tieng CT, Rank RG (1998) Role of NK cells in early host response to chlamydial genital infection. Infect Immun 66: 5867–5873.
31. Colonna M, Brooks EG, Falco M, Ferrara GB, Strominger JL (1993) Generation
of Immunoglobulin-Superfamily C. Science (80-) 260: 1121–1124.

32. Ciccone E, Pende D, Vitale M, Nanni L, Di Donato C, et al. (1994) Self class I killer cell receptors bind to HLA class I molecules. Eur J Immunol 26: 365–369.

33. Stewart CA, Laugier-Anfossi F, Vely F, Saulquin X, Riedmüller J, et al. (2005) KIR and KLR/HLA compound genotypes affect the kinetics of human antiviral natural killer cell responses. J Clin Invest 118: 1017–1026. doi:10.1172/JCI32400 [doi].

34. Ahlenstiel G, Martin MP, Gao X, Carrington M, Rehermann B (2008) Distinct KIR/HLA compound genotypes affect the kinetics of human antiviral natural killer cell responses. J Clin Invest 118: 1017–1026. doi:10.1172/JCI32400 [doi].

35. Horvath S, Xu X, Laird NM (2001) The family based association test method: Facilitation of KIR genotyping by a PCR-SSP method that amplifies short DNA fragments. Tissue Antigens 70: 415–422. doi:10.1111/j.1399-0039.2007.00923.x.

36. Cano P, Klitz W, Mack SJ, Maiers S, Marsh SG, et al. (2007) Common and well-documented HLA alleles: report of the Ad-Hoc committee of the american society for histocompatibility and immunogenetics. Hum Immunol 68: 392–417.

37. Horvath S, Xu X, Laird NM (2001) The family based association test method: Facilitation of KIR genotyping by a PCR-SSP method that amplifies short DNA fragments. Tissue Antigens 70: 415–422. doi:10.1111/j.1399-0039.2007.00923.x.

38. Saftlas AF, Walschmidt M, Lodgen-sackett N, Triche E, Field E (2004) Optimizing buccal cell DNA fields in infants for human leukocyte antigen typing. Pediatr Dev Pathol 7: 144–148. doi:10.1381/pdp.040314.

39. Lu Z, Zhang B, Chen S, Gai Z, Feng Z, et al. (2008) Association of KIR genotypes and haplotypes with susceptibility to chronic hepatitis B virus infection in the Chinese Han population. Cell Mol Immunol 5: 457–463. doi:10.1038/cmi.2008.57.

40. Ahlenstiel G, Martin MP, Gao X, Carrington M, Rehermann B (2008) Distinct KIR/HLA compound genotypes affect the kinetics of human antiviral natural killer cell responses. J Clin Invest 118: 1017–1026. doi:10.1172/JCI32400 [doi].

41. Natividad A, Hanchard N, Holland MJ, Mahdi OSM, Diakite M, et al. (2007) Unusual selection on the KIR3DL1/S1 natural killer cell receptor in sub-Saharan Africans. PLoS Negl Trop Dis 7: e2117. doi:10.1371/journal.pntd.0002117.

42. Van Steen K, Lange C (2005) PBAT: a comprehensive software package for genome-wide association analysis of complex family-based studies. Hum Genomics 2: 67–69.

43. Martin MP, Qi Y, Gao X, Yamada E, Martin JN, et al. (2007) Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. Nat Genet 39: 735–740. doi:10.1038/ng2035.

44. Natividad A, Hanchard N, Holland MJ, Mahdi OSM, Diakite M, et al. (2007) Unusual selection on the KIR3DL1/S1 natural killer cell receptor in sub-Saharan Africans. PLoS Negl Trop Dis 7: e2117. doi:10.1371/journal.pntd.0002117.

45. Khakoo SI, Aiyar A, Quayle AJ, Schust DJ (2012) Modulation of MICA on the Ocular-tissue interface. Graefes Arch Clin Exp Ophthalmol 250: 1144–1154. doi:10.1007/s00417-011-2137-0.

46. Besson C, Roetynck S, Williams F, Orsi L, Amiel C, et al. (2007) Association of KIR3DL1 with susceptibility to Chlamydia trachomatis infection in Gambians. Genes Immun 8: 289–295. doi:10.1038/sj.gene.6364384.

47. Flores-Villanueva PO, Yunnis Ej, Delgado JC, Vittinghoff E, Buchbinder S, et al. (2001) Control of HIV-1 viremia and protection from AIDS are associated with HLA-B*44:01. Proc Natl Acad Sci U S A 98: 5140–5145. doi:10.1073/pnas.07134819.

48. Saftlas AF, Walschmidt M, Lodgen-sackett N, Triche E, Field E (2004) Optimizing buccal cell DNA fields in infants for human leukocyte antigen typing. Pediatr Dev Pathol 7: 144–148. doi:10.1381/pdp.040314.

49. Dolin PJ, Faal H, Johnson GJ, Ajewole J, Mohamed a a, et al. (1998) Trachoma in the Gambia. Br J Ophthalmol 82: 930–933. doi:10.1136/bjo.82.9.930.

50. Van Steen K, Lange C (2005) PBAT: a comprehensive software package for genome-wide association analysis of complex family-based studies. Hum Genomics 2: 67–69.

51. Stare D, Harding-Esch E, Munoz B, Bailey R, Mabey D, et al. (2011) Design and baseline data of a randomized trial to evaluate coverage and frequency of mass treatment with azithromycin: the Partnership for Rapid Elimination of Trachoma (PRET) in Tanzania and The Gambia. Ophthalmic Epidemiol 18: 20–29. doi:10.1016/j.ophtha.2010.05.009.

52. Khakoo SI, Aiyar A, Quayle AJ, Schust DJ (2012) Modulation of MICA on the Ocular-tissue interface. Graefes Arch Clin Exp Ophthalmol 250: 1144–1154. doi:10.1007/s00417-011-2137-0.

53. Khakoo SI, Aiyar A, Quayle AJ, Schust DJ (2012) Modulation of MICA on the Ocular-tissue interface. Graefes Arch Clin Exp Ophthalmol 250: 1144–1154. doi:10.1007/s00417-011-2137-0.

54. Cano P, Klitz W, Mack SJ, Maiers S, Marsh SG, et al. (2007) Common and well-documented HLA alleles: report of the Ad-Hoc committee of the american society for histocompatibility and immunogenetics. Hum Immunol 68: 392–417.

55. Horvath S, Xu X, Laird NM (2001) The family based association test method: Facilitation of KIR genotyping by a PCR-SSP method that amplifies short DNA fragments. Tissue Antigens 70: 415–422. doi:10.1111/j.1399-0039.2007.00923.x.
65. Martin MP, Single RM, Wilson MJ, Tryoudale J, Carrington M (2008) KIR haplotypes defined by segregation analysis in 59 Centre d’Etude Polymorphisme Humain (CEPH) families. Immunogenetics 60: 767–774. doi:10.1007/s00251-008-0334-y.

66. Suarez-Maza A, Steiner QG, Grundschaber C, Tiercy JM (2000) The molecular determination of HLA-Cw alleles in the Mandenka (West Africa) reveals a close genetic relationship between Africans and Europeans. Tissue Antigens 56: 303–312. doi:10.1034/j.1399-0039.2000.560402.x.

67. Maier M, Gragert I, Klitz W (2007). High-resolution HLA alleles and haplotypes in the United States population. Hum Immunol 68: 779–788. doi:10.1016/j.humimm.2007.04.005.

68. Ibanez JA, Schut JH, Sugimoto J, Greene SJ, et al. (2011) Chlamydia trachomatis immune evasion via downregulation of MHC class I surface expression involves direct and indirect mechanisms. Infect Dis Obstet Gynecol 2011: 429095. doi:10.1155/2011/429095.

69. Zheng G, Liu L, Fan P, Ji H, Dong F, Huang Y (2001) Identification of a chlamydial protease-like activity factor responsible for the degradation of host transcription factor RXFs during the inhibition of both constitutive and interferon gamma-inducible major histocompatibility complex class I expression in chlamydia-infected cells. J Exp Med 191: 1525–1534.

70. Zheng G, Fan P, Ji H, Dong F, Huang Y (2001). Identification of a chlamydial protease-like activity factor responsible for the degradation of host transcription factors. J Exp Med 193: 935–942.

71. Korbel DS, Norman PJ, Newman KC, Horowitz A, Gendzekhadze K, et al. (2012) Killer cell immunoglobulin-like receptor gene association with tuberculosis. Hum Immunol 74: 85–92. doi:10.1016/j.humimm.2012.10.006.

72. Leavy O (2013) Natural killer cells: a virtual pick and mix. Nat Rev Immunol 13: 1688–1691. doi:10.1038/nri3566.

73. Lu C, Shen Y-J, Deng Y-F, Wang C-Y, Fan G, et al. (2012) Association of killer immunoglobulin-like receptor with pulmonary tuberculosis in Chinese Han. Genet Mol Res 11: 1370–1378. doi:10.4238/2012.May.15.7.

74. Lu C, Shen Y-J, Deng Y-F, Wang C-Y, Fan G, et al. (2012) Association of killer cell immunoglobulin-like receptors with pulmonary tuberculosis in Chinese Han. Genet Mol Res 11: 1370–1378. doi:10.4238/2012.May.15.7.

75. Horowitz A, Strauss-Albee DM, Leipold M, Kubo J, Nemat-Gorgani N, et al. (2009) Susceptibility to Crohn’s disease is mediated by KIR2DL2/KIR2DL3 heterozygosity and the HLA-C ligand. Immunogenetics 61: 663–671. doi:10.1007/s00251-009-0396-5.

76. Schapiro JM, Segov Y, Rammel B, Pfeffer RM, Davisson T, et al. (2007) Killer cell immunoglobulin-like receptor (KIR) genotype predicts the capacity of human NK cells to respond to pathogen-associated signals. PLoS Pathog 3: e1002042. doi:10.1371/journal.ppat.1002042.

77. Schapiro JM, Segev Y, Rannon L, Alkan M, Rager-Zisman B (1990) Natural killer cell immunoglobulin-like receptors with pulmonary tuberculosis in Chinese Han. Genet Mol Res 11: 1370–1378. doi:10.4238/2012.May.15.7.

78. Horowitz A, Behrens RH, Okell L, Fooks AR, Riley EM (2010) NK cells as adjuvant. J Immunol 184: 4327–4337. doi:10.4049/jimmunol.0903357.

79. Horowitz A, Hafalla J, Meenagh A, Contreras S, Zavaleta R, et al. (2006) Study of KIR genes in tuberculosis patients. Tissue Antigens 68: 306–309. doi:TAN685 [pii] 10.1111/j.1399-0039.2006.00605.x [doi].

80. Schapiro JM, Segov Y, Rammel B, Pfeffer RM, Davisson T, et al. (2007) Killer cell immunoglobulin-like receptor (KIR) genotype predicts the capacity of human NK cells to respond to pathogen-associated signals. PLoS Pathog 3: e1002042. doi:10.1371/journal.ppat.1002042.

81. Mahfouz R, Halas H, Hotell R, Saadeh M, Shamseldin W, et al. (2011) Study of KIR genes in Lebanese patients with tuberculosis. Int J Tuberc Lung Dis 15: 1688–1691. doi:10.5588/ijtld.11.0138.

82. Pydi SS, Sunder SR, VenkataSubramanian S, Kovvali S, Jomnalagada S, et al. (2013) Killer cell immunoglobulin like receptor gene association with tuberculosis. Hum Immunol 74: 85–92. doi:10.1016/j.humimm.2012.10.006.

83. Yindom L-M, Forbes R, Aka P, Janha O, Jeffries D, et al. (2012) Killer-cell immunoglobulin-like receptors and malaria caused by Plasmodium falciparum in The Gambia. Tissue Antigens 79: 104–113. doi:10.1111/j.1399-0039.2011.01818.x.

84. Cates W, Khawaja K, Hanamantachai H, Naka I, et al. (2012) Significant association of KIR2DL3-HLA-C1 combination with cerebral malaria and implications for co-evolution of KIR and HLA. PLoS Pathog 8: e1002563. doi:10.1371/journal.ppat.1002563.

85. Mendez A, Grandu H, Meenagh A, Contreras S, Zavaleta R, et al. (2006) Study of KIR genes in tuberculosis patients. Tissue Antigens 68: 306–309. doi:TAN685 [pii] 10.1111/j.1399-0039.2006.00605.x [doi].

86. Horowitz A, Hafalla J, Meenagh A, Contreras S, Zavaleta R, et al. (2006) Study of KIR genes in tuberculosis patients. Tissue Antigens 68: 306–309. doi:TAN685 [pii] 10.1111/j.1399-0039.2006.00605.x [doi].

87. Horowitz A, Hafalla J, Meenagh A, Contreras S, Zavaleta R, et al. (2006) Study of KIR genes in tuberculosis patients. Tissue Antigens 68: 306–309. doi:TAN685 [pii] 10.1111/j.1399-0039.2006.00605.x [doi].

88. Horowitz A, Behrens RH, Okell L, Fooks AR, Riley EM (2010) NK cells as adjuvant. J Immunol 184: 4327–4337. doi:10.4049/jimmunol.0903357.

89. Horowitz A, Hafalla J, Meenagh A, Contreras S, Zavaleta R, et al. (2006) Study of KIR genes in tuberculosis patients. Tissue Antigens 68: 306–309. doi:TAN685 [pii] 10.1111/j.1399-0039.2006.00605.x [doi].

90. Rolf A, Pollmann J, Cerwenka A (2013) Memory of infections: an emerging role for natural killer cell function in dendritic cell-based vaccines. Expert Rev Vaccines 12: 55–65. doi:10.1586/14760584.12.1.55.