Infection, Genetics and Evolution 29 (2015) 6–14

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Interferon-γ (*IFNG*) microsatellite repeat and single nucleotide polymorphism haplotypes of IFN-α receptor (*IFNAR1*) associated with enhanced malaria susceptibility in Indian populations

Kanika Kanchan [a], Pankaj Jha [b], Sudhanshu S. Pati [c], Sanjib Mohanty [c], Saroj K. Mishra [c], Surya K. Sharma [d], Shally Awasthi [e], Vimala Venkatesh [e], Saman Habib [a,*]

[a] *Division of Molecular and Structural Biology, CSIR-Central Drug Research Institute, Lucknow, India*
[b] *Genomics and Molecular Medicine, CSIR-Institute of Genomics and Integrative Biology, Delhi, India*
[c] *Ispat General Hospital, Rourkela, India*
[d] *National Institute of Malaria Research, New Delhi, India*
[e] *King George's Medical University, Lucknow, India*

ARTICLE INFO

Article history:
Received 31 July 2014
Received in revised form 22 October 2014
Accepted 29 October 2014
Available online 7 November 2014

Keywords:
Malaria susceptibility
Polymorphisms
Cytokine
Receptor
Haplotypes

ABSTRACT

Pro-inflammatory cytokines IFNγ and IFNα function through their cellular receptors IFNγR1 and IFNαR1, respectively to mediate immune processes during malaria infection. A total of 21 SNPs, 2 ins/del polymorphisms and a microsatellite repeat, selected on the basis of their reported association with infectious diseases including malaria in world populations, were analysed for association with *Plasmodium falciparum* malaria susceptibility in a case-control study with adult patients and ethnically-matched controls drawn from a disease meso- to hyperendemic and a nonendemic region of India. Among the five *IFNG* SNPs tested, an intron 3 and a 3′UTR SNP associated with disease in the endemic region. In addition, large $(CA)_n$ repeats of *IFNG* intron 1 associated with protection from severe malaria in the endemic region (severe vs. control, odds ratio = 0.21, 95% CI = 0.08–0.52, $P = 1.3 \times 10^{-4}$). The TA11CAG haplotype (rs2069705 T/C, rs2430561 A/T, rs3138557 $(CA)_n$, rs2069718 T/C, rs2069727 A/G, rs2069728 G/A) carrying a short CA_{11} repeat also exhibited very strong association with severe malaria, particularly in the endemic region (severe vs. control, OR = 14.56, 95% CI = 3.39–85.81, $P = 3 \times 10^{-5}$). One SNP each from the *IFNA8* and *IFNA17* of *IFNA* gene cluster had a protective effect in the non-endemic region but not in the endemic region. A promoter and an intron 2 SNP of *IFNAR1* were risk factors for disease and the *IFNAR1* haplotype GCCAGG (rs2843710 C/G, rs2850015 C/T, +6993 C/T, rs2243594 A/G, rs1012335 G/C, rs2257167 G/C) carrying both the risk alleles strikingly associated with disease manifestation in the endemic region (severe vs. control, OR = 27.14, 95% CI = 3.12–1254, $P = 2 \times 10^{-5}$; non-severe vs. control, OR = 61.87, 95% CI = 10.08–2521, $P = 1 \times 10^{-8}$). The data indicates dissimilar contribution of cytokine and cytokine receptor variants to disease in populations residing in areas of differential malaria endemicity.

1. Introduction

Malaria continues to be one of the major infectious parasitic diseases affecting humans globally (World Malaria Report, 2013). Apart from factors such as parasite virulence, drug resistance, transmission intensity, and environmental conditions, genetic factors of the human host also contribute to susceptibility/resistance to disease. Several reports suggest evidence of ethnic differences in susceptibility to malaria (Mendonca et al., 2012) and polymorphisms in more than 30 genes have been associated with disease in different world populations (Kwiatkowski, 2005). One aspect of severe malaria pathogenesis is an excessive or dysregulated inflammatory response to infection (Clark et al., 1987; Day et al., 1999; Lyke et al., 2004; Sinha et al., 2008a). Inflammatory cytokines have been shown to play an important role in immune response to malaria with the balance between pro- and anti-inflammatory cytokines skewed towards the former resulting in exacerbated inflammatory responses (Clark et al., 2006; Clark and Cowden, 2003; Sinha et al., 2010). Cytokines exert their effects through cognate cell-surface receptor molecules. Variations within promoter or other regulatory sequences of cytokine genes may

---

* Corresponding author at: Division of Molecular and Structural Biology, CSIR-Central Drug Research Institute, Sector 10, Jankipuram Extension, Sitapur Road, Lucknow 226031, India. Tel.: +91 522 2772477; fax: +91 522 2771941.

E-mail addresses: saman.habib@gmail.com, saman_habib@cdri.res.in (S. Habib).

http://dx.doi.org/10.1016/j.meegid.2014.10.030
1567-1348/© 2014 Elsevier B.V. All rights reserved.

directly influence the levels of cytokines; genetic polymorphisms in cytokine receptors may result in altered expression levels of the protein or expression of an abnormal protein with compromised affinity to the cognate cytokine resulting in impaired signalling (Newport et al., 2004).

IFNγ is a key immunological mediator that has a protective role in host defence against malaria, but also contributes to underlying disease pathology (Stevenson and Riley, 2004). The target cells of IFNγ during *Plasmodium falciparum* infection are monocytes/macrophages (Bate et al., 1988), neutrophils (Kumaratilake et al., 1991), Th2 cells (Taverne, 1993) and parasite-infected hepatocytes (Klotz et al., 1995). IFNγ also serves as a macrophage activating factor involved in innate immune response to malaria (Malaguarnera and Musumeci, 2002). IFNγ activated macrophages release tumour necrosis factor α (TNF), transforming growth factor β (TGFβ), interleukins IL-1 and IL-6, and reactive oxygen and nitrogen radicals (Clark et al., 1997). Additionally, IFNγ activates iNOS and induces the L-arginine dependent NO pathway via signal transducers associated with transcription. This NO pathway leads to the subsequent elimination of the infected hepatocytes (Snounou et al., 2000). IFNγ induces cellular activation by binding to a receptor complex – the interferon gamma receptor (IFNγR). IFNγR is ubiquitously expressed on all monocytes, macrophages, T cells, B cells, NK cells, neutrophils, fibroblast and endothelial cells (Juliger et al., 2003). The receptor is a heterodimer of two subunits, α and β, that are integral membrane proteins. Subunit α (IFNγR1) is essential for IFNγ binding, receptor trafficking, and signal transduction (Bach et al., 1997) whereas the β subunit (IFNγR2) functions as a transmembrane accessory factor. The heterodimeric IFNγR interacts with STAT1, GAF, JAK1, and/or JAK2 in the downstream IFNγ signal transduction pathway (Larkin et al., 2000).

Several polymorphisms (SNPs and microsatellite repeat) from the promoter, introns and UTR region of the *IFNG* gene have been reported (Koch et al., 2005). Some of these polymorphisms have been associated with diseases other than malaria in different world populations (Ding et al., 2008; Kim et al., 2012). Koch et al. (2005) have identified a weak association of two *IFNG* SNPs with severe malarial anaemia and cerebral malaria in Mandinka, the largest ethnic group in Gambia. Mutations in the *IFNGR1* gene have also been reported to cause extreme vulnerability to infection with *Mycobacterium tuberculosis* (He et al., 2010), leishmaniasis (Salih et al., 2007) and *Salmonella* (Altare et al., 1998). Several polymorphisms in the promoter region of *IFNGR1* which affect gene regulation have also been reported to influence susceptibility to severe malaria in the Mandinka (Koch et al., 2002).

There is a possible signalling connection between IFNα and IFNγ, where IFNα is thought to act upstream of IFNγ (Cousens et al., 1999; Haque et al., 2011; Takaoka et al., 2000). Although the role of IFNα in *P. falciparum* malaria is largely unexplored, its involvement in inducing an increased immune response against malarial antigens has been reported (Sturchler et al., 1989). Studies on murine models of malaria have demonstrated that treatment with recombinant human IFNα resulted in reduced blood parasite levels and mortality from cerebral malaria, but had no effect on anaemia outcomes (Vigario et al., 2001, 2007). Additionally, it has also been reported that reduced circulating IFNα levels are associated with severe falciparum malaria in Gabonese (Luty et al., 2000) and Kenyan (Ong'echa et al., 2011) children. IFNα exerts its effect by binding to IFNαR. IFNαR is composed of two membrane spanning proteins, IFNαR1 and IFNαR2, that form a ternary complex with the ligand (Meyer, 2009). As IFNαR1 is expressed on virtually every cell type (Ito et al., 2006; Prinz et al., 2008), IFNα is able to mediate a vast effect on the immune response.

There are 13 *IFNA* intron-less genes clustered on human chromosome 9, encoding for 13 mature proteins (Samuel, 2001). However, post-translational modifications result in more than 22 IFNα subtypes (Bekisz et al., 2004; Song le et al., 2006). A considerable number of exonic and 5′ and 3′ flanking region polymorphisms exist among the *IFNA* genes. A promoter polymorphism each of *IFNA2* and *IFNA8* has recently been shown to be associated with reduced IFNα production and increased susceptibility to severe malarial anaemia and mortality in Kenyan populations (Kempaiah et al., 2012). Association of *IFNAR1* variants with protection against severe and cerebral malaria in African and South East Asian populations has also been reported (Aucan et al., 2003; Ball et al., 2013; Khor et al., 2007).

The possible involvement of polymorphisms in *IFNG*, *IFNGR1*, *IFNA* gene cluster and *IFNAR*1 genes in resistance/susceptibility to *P. falciparum* malaria in the genetically diverse Indian populations has not been investigated. We explored the possible association of 24 polymorphisms (21 SNPs, 2 ins/del polymorphisms and a microsatellite repeat) in the two cytokine genes and their receptors with falciparum malaria in populations residing in a malaria endemic and a non-endemic region of India and report differential effects of selected individual variants and haplotypes on disease outcome.

2. Materials and methods

2.1. Study subjects

This study was carried out on the existing panel of patient and control samples collected from a *P. falciparum* endemic (Antagarh, Chhattisgarh and Sundargarh, Orissa) and a non-endemic (Lucknow and surrounding areas of Uttar Pradesh) region of India as described earlier (Jha et al., 2012; Sinha et al., 2008a,b). The tribal groups in the endemic region are of the Austro-Asiatic and Dravidian linguistic lineages and patients and controls in the non-endemic region are from large populations (caste and religious groups) of the Indo-European lineage. The control panel comprised of individuals above the age of 18 years ethnically-matched with the patient group, and belonging to the Bhumij, Munda, Oraon and Gond tribal populations in the meso to hyperendemic region of the bordering states of Chhattisgarh and Orissa (Kumar et al., 2007). There is preponderance of *P. falciparum* of >90% in this region. Malaria transmission in Sundargarh is through *Anopheles fluviatilis* and *Anopheles culicifacies* (Sharma et al., 2006). *A. fluviatilis* has an entomological inoculation rate (EIR) of 0.23–0.39 infective bites per person per day during the high transmission season and *A. culicifacies* has an EIR of 0.01 infective bites per person per day in this region (Shah et al., 2013). Ethnically-matched patient and control groups of the non-endemic region were from the Aggarwal, Brahmin, Kayastha, Pasi, Thakur, Yadav, Shia and Sunni caste and religious groups. Ethical clearance for this study was obtained from participating institutes/hospitals, and informed consent was obtained from each volunteer/guardian. The samples comprised a total of 221 *P. falciparum* adult malaria patients [101 from endemic (average age 31 ± 13.5 years) and 120 from non-endemic region (average age 24.75 ± 15.8 years)] and 192 control samples [102 from endemic (average age 38.9 ± 12.6 years) 90 from non-endemic (average age 28.7 ± 3.9 years) region] from ethnically-matched and unrelated individuals (Jha et al., 2012; Sinha et al., 2008a). WHO guidelines (WHO, 2000) were followed to categorise severe and non-severe malaria as described previously (Jha et al., 2012; Sinha et al., 2008a). Non-severe patients were *P. falciparum* positive in a rapid diagnostic test (Optimal/Paracheck) readout and blood smear, had fever and lacked symptoms that characterised severe malaria. Severe malaria patients were characterised by any of the following symptoms together with fever: impaired consciousness (coma), severe anaemia, acidotic breathing, pulmonary edema, increased serum creatinine, and increased bilirubin levels. All controls recruited were healthy individuals with no reported history of