Infection, Genetics and Evolution 40 (2016) 162–166

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Contribution of inflammasome genetics in *Plasmodium vivax* malaria

Marina L.S. Santos [a], Edione Cristina Reis [b], Pamela N. Bricher [b], Tais N. Sousa [a], Cristiana F.A. Brito [a], Marcus V.G. Lacerda [c], Cor J.F. Fontes [d], Luzia H. Carvalho [a], Alessandra Pontillo [b,*]

[a] Laboratório de Malária, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, MG, Brazil
[b] Laboratório de Imunogenética, Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brazil
[c] Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Instituto de Pesquisas Leônidas and Maria Deane, Fundação Oswaldo Cruz, Manaus, AM, Brazil
[d] Hospital Julio Muller, Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil

ARTICLE INFO

Article history:
Received 1 December 2015
Received in revised form 26 February 2016
Accepted 28 February 2016
Available online 3 March 2016

Keywords:
Inflammasome
NLRP1
NLRP3
IL1B
IL18
P. vivax
Malaria

ABSTRACT

Recent reports showed that, in mice, symptomatic *Plasmodium* infection triggers NLRP3/NLRP12-dependent inflammasome formation and caspase-1 activation in monocytes. In humans, few works demonstrated that inflammasome is activated in malaria. As *Plasmodium vivax* is a potent inducer of inflammatory response we hypothesised that inflammasome genetics might affect *P. vivax* malaria clinical presentation. For this purpose, selected SNPs in inflammasome genes were analysed among patients with symptomatic *P. vivax* malaria.

157 Brazilian Amazon patients with *P. vivax* malaria were genotyped for 10 single nucleotide polymorphisms (SNPs) in inflammasome genes *NLRP1*, *NLRP3*, *AIM2*, *CARD8*, *IL1B*, *IL18* and *MEFV*. Effect of SNPs on hematologic and clinical parameters was analysed by multivariate analysis.

Our data suggested an important role of NLRP1 inflammasome receptor in shaping the clinical presentation of *P. vivax* malaria, in term of presence of fever, anaemia and thrombocytopenia. Moreover *IL1B* rs1143634 resulted significantly associated to patients' parasitaemia, while *IL18* rs5744256 plays a protective role against the development of anaemia.

Polymorphisms in inflammasome genes could affect one or other aspects of malaria pathogenesis. Moreover, these data reveal novel aspects of *P. vivax*/host interaction that involved NLRP1-inflammasome.

1. Background

Malaria remains a major public health problem worldwide, with 3.2 billion people at risk of being infected and developing the disease (WHO, 2015). Among the five *Plasmodium* species that cause malaria in humans, *Plasmodium falciparum* has been considered the main cause of severe and fatal disease, while *P. vivax* is a major cause of morbidity outside Africa and has also been associated with clinical severity and systemic inflammation (Barber et al., 2015).

A central mechanism for inflammation induction is the secretion of pro-inflammatory cytokines IL-1ß e IL-18 from innate immune cells. IL-1ß e IL-18 liberation depends on the activation of a cytoplasmic complex, known as inflammasome. Several intracellular Pattern Recognition Receptors (PRRs) belonging to NLR family (i.e.: NLRP1, NLRP3, NLRP12) and to other protein families (i.e.: AIM2 or pyrin/MEFV), are able to induce the inflammasome assembling in response to pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs). Upon recognition of PAMPs and/or DAMPs, the receptor recruits the adaptor protein ASC and the cysteine-aspartic protease caspase-1, which is responsible for the processing of pro-IL-1ß and pro-IL-18 (Man and Kanneganti, 2015).

Recent reports showed that, in mice, symptomatic *Plasmodium* infection triggers NLRP3/NLRP12-dependent inflammasome formation and caspase-1 activation in monocytes, leading to dramatic IL-1ß secretion especially when exposed to a second microbial challenge (Ataide et al., 2014). Furthermore hemozoin and DNA activated inflammasome through NLRP3 and AIM2 receptors respectively, in murine infected erythrocytes (Kalantari et al., 2014).

In humans, few works demonstrated that inflammasome is activated in malaria. In *P. falciparum*, for example, the opsonization of malaria-infected erythrocytes activates the inflammasome and leads to FcR-mediated phagocytosis in macrophages (Zhou et al., 2012). On the other hand, the presence of inflammasome complexes was demonstrated in monocytes from malaria patients containing either NLRP3 or NLRP12 inflammasomes (Ataide et al., 2014).

Although multiple studies have revealed that malaria has been a major force of evolutionary selection on the human genome (reviewed in (Malaria Genomic Epidemiology, 2014) little is currently known about the effects of malaria on the evolution of the human immune genes, possibly because the phenotypic consequences are more subtle than those of the classic erythrocyte variants (Kwiatkowski, 2005).

* Corresponding author at: Laboratório de Imunogenética, Departamento de Imunologia, Instituto de Ciências Biomédicas, Avenida Prof. Lineu Prestes, 1730, Cidade Universitária, São Paulo, SP, Brazil.
E-mail address: alepontillo@usp.br (A. Pontillo).

http://dx.doi.org/10.1016/j.meegid.2016.02.038
1567-1348/

Table 1
Demographic, clinical and haematological data of Brazilian Amazon patients with *P. vivax* malaria.

CHARACTERISTICS	
Median age, years	34 (25–80)
Male/female, n	122/35
Previous malaria episodes[§], n	1.5 (1–40)
Days of acute illness, n	5 (3–7)
Presence of fever, yes/no	134/23
Parasitaemia, parasites/μL of blood	1117 (607.5–8238)
Leukocyte counts, $\times 10^3$/μl	5.4 (4.4–14.6)
Haematocrit, %	38.8 (35.6–50.6)
Haemoglobin level, g/dL	Male: 13.2 (12.1–16.9); Female: 12.3 (11.3–15.2)
Anaemia[¥], yes/no	Male: 52/70; Female:12/23
Platelet counts, $\times 10^3$/μl	95 (62–260)
Thrombocytopenia[‡], yes/no	23/134
Total score, high/low	93/64

Data are reported as median (interquartile range) except where otherwise specified.
[§] Self-reported number of lifetime malaria episodes.
[¥] Anaemia: haemoglobin <13 g/dL (Male) and <12 g/dL (Female).
[‡] Thrombocytopenia: platelet counts $<50 \times 10^3$/μL.

Notwithstanding, the last few years have seen a rapid growth in the number of reported genetic associations with susceptibility and resistance to clinical malaria, many of which involve immunity and inflammation. In this context, genetic variants in host innate immune genes (i.e., *CRP*, *MBL2*, *NOS2*, *IFNAR1*) and adhesion molecules (*THBS1* and *ESEL*) have been described as predisposing factors for the outcome of *P. falciparum* malaria (Kanchan et al., 2015a, 2015b), while antioxidant enzymes (i.e.: *GSTT*, *GSTM*, *GSTP*, *SOD*, *CAT*) were evaluated as risk factors for *P. vivax* malaria (Andrade et al., 2010; Fernandes et al., 2015).

As *P. vivax* is a potent inducer of inflammatory response (reviewed in (Anstey et al., 2009) we hypothesised that inflammasome genetics might affect *P. vivax* malaria clinical presentation. To our knowledge, inflammasome genes have not yet been evaluated in malaria. For this purpose, selected single nucleotide polymorphisms (SNPs) in inflammasome genes were analysed among Brazilian Amazon patients with symptomatic *P. vivax* malaria.

2. Material and methods

2.1. Subjects

A total of 157 patients with *P. vivax* malaria (122 males/35 females) were recruited for the study after written informed consent, as specified by the Brazilian National Commission for Ethics on Research (Protocol CEPSH/CPqRR/03/2008). Antimalarial and supportive therapies were given according to standard protocols. The study included patients with symptomatic *P. vivax* malaria, and all volunteers were negative for *P. falciparum* and/or *P. malariae* by the means of microscopy and polymerase chain reaction (PCR). Clinical and demographical data were acquired through a standardized questionnaire, and the haematological profiles were assessed by automated complete blood cell counts carried out at local health facilities. Table 1 summarizes demographic, epidemiological, parasitological and haematological data of *P. vivax* infected-volunteers. For statistical purposes, we used a previously validated semi-quantitative clinical assessment to enable numerical comparisons (Campos et al., 2013). Briefly, scores of 0 or 1 were assigned to clinical and hematologic parameters reported as absent (or within reference ranges) or present (or outside reference ranges), respectively; the sum of scores provided patient's final clinical score (scores range from 0 to 7). We grouped 0–3 score as "low" and 4–7 as "high" total clinical score.

2.2. Single nucleotide polymorphisms selection and genotyping

10 SNPs in inflammasome genes *NLRP1*, *NLRP3*, *MEFV*, *CARD8*, *IL1B*, and *IL18* were selected based on functional effect, minor allele frequency (MAF) and/or previously reported association with human disorders (Levandowski et al., 2013; Verma et al., 2012) (Frayling et al., 2007; Hitomi et al., 2009; Pontillo et al., 2011, 2012a, 2012b, 2013). SNPs genotyping was performed using commercially available TaqMan assays (Applied Biosystems/AB) using StepOne Real-Time platform (AB). Allelic discrimination was performed using the StepOne software (AB).

2.3. Data analysis

Effect of SNPs on hematologic and clinical parameters, including haematocrit, haemoglobin, leukocyte and platelet levels, parasitaemia, fever, presence of severe thrombocytopenia and anaemia as well as the total clinical score was analysed by multivariate association based

Table 2
Frequency of studied polymorphisms in Brazilian Amazon cohort of patients with *P. vivax* malaria.

Gene	SNP ID	Alleles/ Genotypes	Frequency	H-W p-value
NLRP1	rs12150220	A	0.41	0.185
		T	0.59	
		A/A	0.38	
		A/T	0.43	
		T/T	0.19	
	rs11651270	C	0.48	0.247
		T	0.52	
		C/C	0.25	
		C/T	0.45	
		T/T	0.30	
	rs2670660	A	0.46	0.743
		G	0.54	
		A/A	0.28	
		A/G	0.52	
		G/G	0.20	
NLRP3	rs35829419	A	0.02	1.0
		C	0.98	
		A/A	0	
		A/C	0.03	
		C/C	0.97	
	rs10754558	C	0.62	0.862
		G	0.38	
		C/C	0.39	
		C/G	0.46	
		G/G	0.15	
MEFV	rs224204	A	0.12	1.0
		G	0.88	
		A/A	0	
		A/G	0.24	
		G/G	0.76	
CARD8	rs2043211	A	0.70	0.556
		T	0.30	
		A/A	0.50	
		A/T	0.40	
		T/T	0.10	
	rs6509365	A	0.63	0.303
		G	0.37	
		A/A	0.37	
		A/G	0.51	
		G/G	0.12	
IL1B	rs1143634	A	0.22	0.218
		G	0.78	
		A/A	0.06	
		A/G	0.30	
		G/G	0.64	
IL18	rs5744256	A	0.83	0.767
		G	0.17	
		A/A	0.69	
		A/G	0.29	
		G/G	0.02	

Gene symbol, Single nucleotide polymorphism (SNP) identification (ID), allele and genotype frequencies and p-value for Hardy–Weinberg (H–W) equilibrium test are reported.