International Journal for Parasitology 43 (2013) 11–15

Contents lists available at SciVerse ScienceDirect

International Journal for Parasitology

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

Succinctus

The role of platelet-activating factor receptor (PAFR) in lung pathology during experimental malaria

Norinne Lacerda-Queiroz [a,*], Milene Alvarenga Rachid [a], Mauro Martins Teixeira [a], Antonio Lucio Teixeira [a,b,*]

[a] Laboratório de Imunofarmacologia/Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, Minas Gerais, Brazil
[b] Departamento de Clínica Médica da Faculdade de Medicina, Universidade Federal de Minas Gerais, Minas Gerais, Brazil

ARTICLE INFO

Article history:
Received 3 September 2012
Received in revised form 28 October 2012
Accepted 19 November 2012
Available online 20 December 2012

Keywords:
Malaria
Lung
Platelet-activating factor receptor
Mouse model
Plasmodium berghei ANKA

ABSTRACT

Malaria-associated lung pathology has been a neglected area in the study of malaria complications. Platelet-activating factor (PAF) is an inflammatory mediator involved in lung inflammation. Using mice lacking the PAF receptor ($PAFR^{-/-}$) we investigated the relevance of signaling through the PAFR for the lung inflammatory process triggered by *Plasmodium berghei* ANKA (PbA) strain infection. In $PAFR^{-/-}$ mice, pulmonary inflammation was markedly reduced as demonstrated by histology, production of certain pro-inflammatory mediators, accumulation of macrophage and CD8+ T cells in the lung parenchyma and the virtual absence of changes in vascular permeability. Therefore, PAFR activation is crucial in the pathogenesis of pulmonary damage associated with PbA infection in C57Bl/6 mice.

Malaria infection may present different clinical forms from asymptomatic to complicated forms, called severe malaria. Severe malaria is a consequence of *Plasmodium falciparum* infection and is characterized by severe anemia, lung injury, acidosis and cerebral malaria (Miller et al., 2002). Lung injury in the context of severe malaria has been a neglected area of study (Mohan et al., 2008). Experimental models using mouse strains infected with *Plasmodium berghei* ANKA (PbA) strain demonstrated malaria-associated lung pathology (Senaldi et al., 1994; Chang et al., 2001; Lovegrove et al., 2008; Fauconnier et al., 2011).

Platelet-activating factor (PAF) is a mediator of the inflammatory response shown to be relevant for several aspects of lung inflammation, including leukocyte influx and increase in vascular permeability (Camussi et al., 1983; Miotla et al., 1998; Uhlig et al., 2005). We previously showed that the absence of PAF receptor (PAFR) protected mice against experimental cerebral malaria (ECM) after PbA infection (Lacerda-Queiroz et al., 2012). Akin to the events in the brain of infected mice lacking PAFR ($PAFR^{-/-}$), a series of experiments were undertaken to assess whether these mice would be protected from lung injury associated with PbA infection.

All experiments were approved by the Animal Ethics Committee of the Federal University of Minas Gerais (UFMG), Brazil. Female C57Bl/6 (wild type, WT) mice, aged 8 weeks, were obtained from Animal Care Facilities of the Institute of Biological Sciences, UFMG. $PAFR^{-/-}$ mice on C57Bl/6 background were bred and maintained under specific pathogen-free conditions. The cloned PbA line used in this study was a kind donation by Dr. Leonardo Carvalho (IOC-Fiocruz, Rio de Janeiro, Brazil) (Blanco et al., 2008; Martins et al., 2009). Mice were infected with PbA by i.p. injection of 10^6 parasitized red blood cells suspended in 0.2 ml of PBS (Grau et al., 1986). On day 6, mice were anesthetized and blood was obtained from the brachial plexus from control and infected mice, and the serum was stored at 20 °C until further analysis. Lungs were immediately removed and one portion was homogenized in extraction solution containing aprotinin. The concentrations of IFN-γ and CC motif chemokine ligand 5/regulated and normal T cell expressed and secreted (CCL5/RANTES) were assessed in the lung and TNF-α, IL-10, CXC motif chemokine ligand 10/IFN-γ -induced protein 10 CXCL10/IP-10, IFN-γ, vascular endothelial growth factor (VEGF) and IL-6 levels were measured in the serum by ELISA (R&D Systems, Minneapolis, MN, USA). For histological analysis, another portion of the lung was preserved in 10% buffered formalin. The sections (4 μm) were stained with H&E, and later examined and scored as previously described (Fauconnier et al., 2011). Digital images were acquired for documentation.

As the administration of PAF has been associated with increased vascular permeability (Miotla et al., 1998; Stafforini et al., 2003),

* Corresponding authors. Address: Laboratory of Immunopharmacology, sala O4 202, Instituto de Ciências Biológicas Av., Antônio Carlos, 6627 Pampulha, Belo Horizonte, MG, Brazil. Tel.: +55 31 3409 2649; fax: +55 31 3409 2545.
E-mail addresses: norinneq@yahoo.com.br (N. Lacerda-Queiroz), altexr@gmail.com (A.L. Teixeira).

0020-7519/$36.00
http://dx.doi.org/10.1016/j.ijpara.2012.11.008

lung integrity was determined by measuring Evans blue (EB) extravasation into the pulmonary parenchyma, as described by Epiphanio et al. (2010). The amount of EB in tissue extracts was measured by absorbance at 610 nm. Results are shown as the amount of EB (pg) present in 100 mg of tissue.

For pulmonary-sequestered cell isolation, mice were euthanized on day 7 p.i, a time point when death occurs for WT mice. The mice were perfused intracardially with PBS and the lungs were removed for leukocyte isolation (Garcia et al., 2010). Briefly, lungs were collected in RPMI 1640 medium (Sigma Chemicals, St. Louis, MO, USA) and incubated in collagenase IV ((Sigma Chemicals) 100 U/mL in RPMI 1640 medium) at 37 °C for 45 min. Digested lung tissue was gently disrupted by passages through a nylon cell strainer (70 μm; Becton Dickinson, Brazil) and then centrifuged at 400*g* for 10 min. The pellet was resuspended in 35% Percoll gradient (Sigma–Aldrich, St. Louis, MO, USA) and this was deposited on a 70% Percoll gradient. After centrifugation (1,100*g*), leukocytes were collected from the boundary layer. Afterwards, leucocytes were resuspended in FACS buffer (PBS containing 1% FCS and 0.01% NaN_3) and counted.

Leucocytes were stained using monoclonal antibodies (mAb) and isotype controls to type different populations. For each population of interest, 50,000 cells were scored. The frequency of positive cells was analyzed and limits for the quadrant markers were always set based on negative population and isotype controls. Data were acquired on a FACScan (Becton Dickinson, San José, CA, USA) and analyzed using FlowJo 7.5.3 software (TreeStar Inc., Ashland, Oregon, USA). Results are presented as absolute number of cells per lung.

Data are shown as mean ± SD and a one-way ANOVA with Newman-Keuls post-test was used for multiple comparisons of data with a normal distribution. Semiquantitative analysis (histopathological score) was performed based on the non-parametric Mann–Whitney *U* test and the data are shown as mean ± S.E.M. Statistical significance was set at $P < 0.05$.

No lesions were detected in the lungs of animals from the non-infected groups. On day 6 p.i., major histopathological changes in the lungs of infected WT mice were characterized by interstitial edema, thickening of alveolar septae, with accumulation of mononuclear cell, and reduction in size of alveoli (Fig. 1A (* and arrow)).

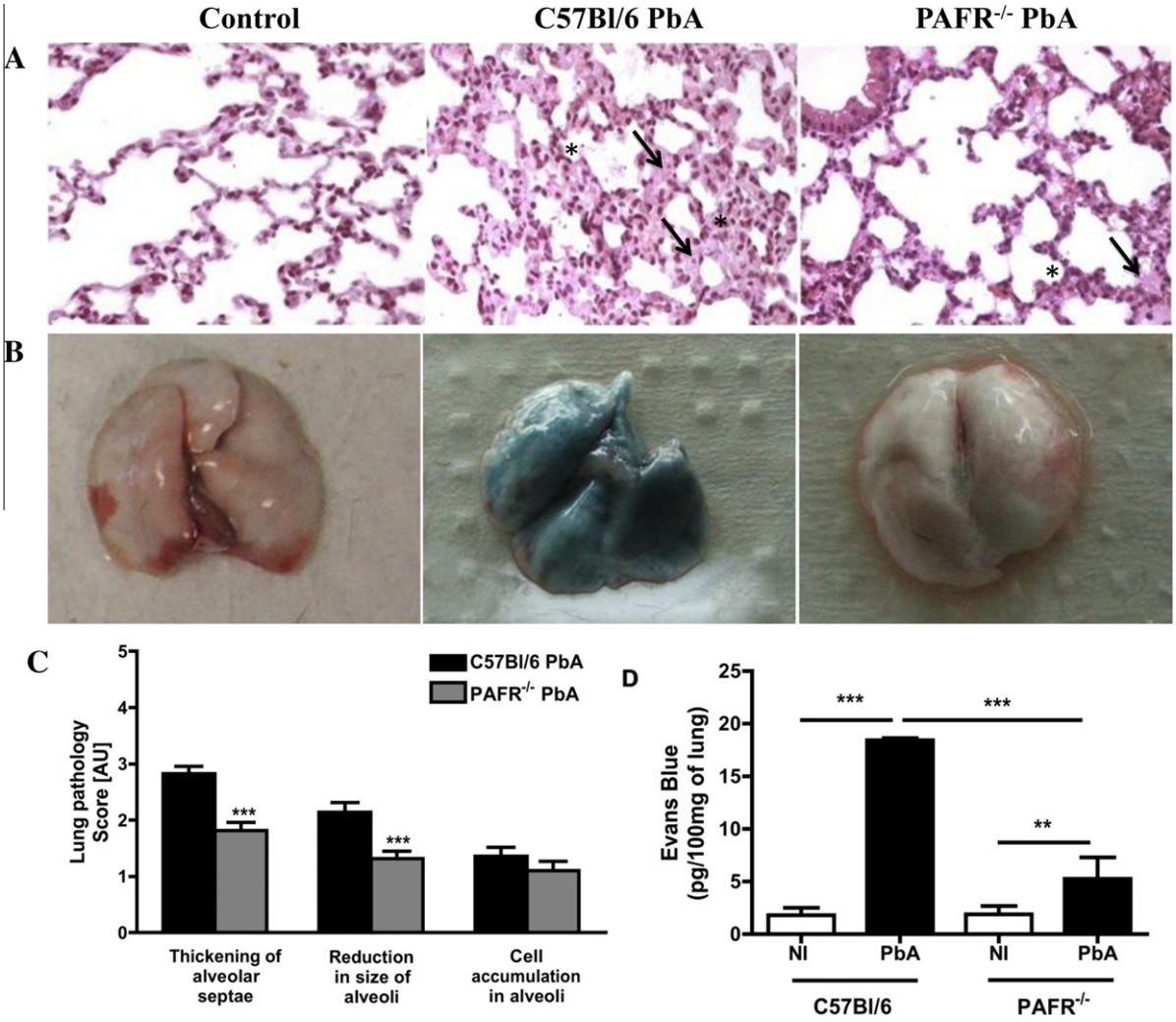

Fig. 1. *Plasmodium berghei* ANKA strain (PbA)-infected mice lacking platelet activating factor receptor (PAFR−/−) present mild histopathological changes in the lung and virtual absence of vascular leakage. (A) Representative photomicrographs (×400) of H&E)-stained lung sections. Normal histological section of lung parenchyma with standard architecture from control (wild type, WT; non-infected, NI) C57Bl/6 mice. PbA-infected C57Bl/6 mice showing slight interstitial edemas (arrows), thickening of alveolar septae (*), with accumulation of mononuclear cells and a reduction in size of alveoli. In contrast, PbA-infected $PAFR^{-/-}$ mice exhibited a less intense edema, thickening of alveolar septae and reduction in the sizes of alveoli. (B) Qualitative assessment of lung vascular permeability using Evans blue extravasation. PbA-infected C57Bl/6 mice exhibited intense discoloration of the lung, whereas control mice and infected $PAFR^{-/-}$ mice demonstrated no discoloration. (C) Semi-quantification score for histopathological changes in the lung of PbA-infected mice (C57Bl/6 and $PAFR^{-/-}$). (D) Quantitative assessment of vascular permeability using Evans blue extravasation in lung extract. Significant differences are indicated by $^{**}P < 0.01$ and $^{***}P < 0.001$, compared with control counterparts or compared with infected WT mice.