Infection, Genetics and Evolution 20 (2013) 270–275

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Short communication

Quantifying variation in the potential for antibody-mediated apparent competition among nine genotypes of the rodent malaria parasite *Plasmodium chabaudi* ☆

Karen J. Fairlie-Clarke [a,*], Judith E. Allen [a], Andrew F. Read [b], Andrea L. Graham [c]

[a] Institutes of Evolution, Immunology and Infection Research, School of Biological Sciences, King's Buildings, University of Edinburgh, Edinburgh, UK
[b] Centre for Infectious Disease Dynamics, Department of Biology, Pennsylvania State University, University Park, PA, USA
[c] Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, USA

ARTICLE INFO

Article history:
Received 4 July 2013
Received in revised form 10 September 2013
Accepted 11 September 2013
Available online 18 September 2013

Keywords:
Plasmodium chabaudi
Cross-reactivity
Within-host competition
Antibody
Mixed infection
Virulence evolution

ABSTRACT

Within-host competition among parasite genotypes affects epidemiology as well as the evolution of virulence. In the rodent malaria *Plasmodium chabaudi*, competition among genotypes, as well as clone-specific and clone-transcending immunity are well documented. However, variation among genotypes in the induction of antibodies is not well understood, despite the important role of antibodies in the clearance of malaria infection. Here, we quantify the potential for antibodies induced by one clone to bind another (i.e., to cause antibody-mediated apparent competition) for nine genetically distinct *P. chabaudi* clones. We hypothesised that clones would vary in the strength of antibody induction, and that the propensity for clone-transcending immunity between a pair of clones would increase with increasing genetic relatedness at key antigenic loci. Using serum collected from mice 35 days post-infection, we measured titres of antibody to an unrelated antigen, Keyhole Limpet Haemocyanin (KLH), and two malaria antigens: recombinant Apical Membrane Antigen-1 (AMA-1) and Merozoite Surface Protein-1_{19} (MSP-1_{19}). Amino acid sequence homology within each antigenic locus was used as a measure of relatedness. We found significant parasite genetic variation for the strength of antibody induction. We also found that relatedness at MSP-1_{19} but not AMA-1 predicted clone-transcending binding. Our results help explain the outcome of chronic-phase mixed infections and generate testable predictions about the pairwise competitive ability of *P. chabaudi* clones.

1. Introduction

Intraspecific competition among parasites in mixed-genotype infections is expected to affect the evolution of parasite traits and of virulence (degree of harm done to hosts) (Mideo, 2009). Such within-host competition has been demonstrated in a wide range of parasite taxa (e.g., (Balmer et al., 2009; Bashey et al., 2013; Hall and Little, 2013)) and can affect establishment of infection and transmission from the host (Karvonen et al., 2011), the virulence of infection (Balmer et al., 2009) and parasite population structure (Gold et al., 2009). Natural malaria infections often comprise more than one genotype per species (Babiker et al., 1994; Mobegi et al., 2012; Read and Taylor, 2001; Vardo and Schall, 2007). The rodent malaria parasite *Plasmodium chabaudi* has been used to investigate the ecological mechanisms of within-host competition (Bell et al., 2006; de Roode et al., 2005a,b; De Roode et al., 2003; Taylor et al., 1997). For example, direct competition for red blood cells (RBCs) is paramount during the acute phase of infection where parasite population growth is exponential (De Roode et al., 2003). However, parasite dynamics during mixed infection are not always easily explained by resource (exploitation) competition, particularly during the chronic phase (e.g., (Bell et al., 2006; Mideo et al., 2008)). Instead, immune-mediated apparent competition (where one genotype induces an immune response capable of targeting other genotypes; e.g., (Jarra and Brown, 1985)) or facilitation (if one genotype distracts immunological attention from others) may determine the outcome of within-host competition (Barclay et al., 2008; Raberg et al., 2006). Importantly, the direction of natural selection on parasite virulence depends upon the mechanism of competition (Mideo, 2009).

Malaria poses a particularly interesting system for considering immune-mediated apparent competition and facilitation, because mammalian adaptive immunity is capable of exquisite specificity to malaria antigens (Couper et al., 2005; Quin and Langhorne,

* Corresponding author. Current address: Strathclyde Institute of Pharmacy and Biomedical Sciences, Strathclyde University, 161 Cathedral Street, Glasgow G4 0RE, UK. Tel.: +44 141 548 4645.
E-mail address: karen.fairlie-clarke@strath.ac.uk (K.J. Fairlie-Clarke).

1567-1348/$ - see front matter © 2013 The Authors. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.meegid.2013.09.013

2001), including species- and strain-specific immunity (Jarra and Brown, 1985, 1989; Martinelli et al., 2005; Pattaradilokrat et al., 2007), yet the parasites also induce cross-reactive antibodies through polyclonal expansion of B-cells. This proliferation and differentiation of B-cells regardless of their antigen-specificity (Montes et al., 2007) is attributed to disruption of spleen architecture, innate activation of B-cells, and induction of cytokine storms (Achtman et al., 2003; Castillo-Mendez et al., 2007; Muxel et al., 2011). Indeed, induction of cross-reactive immune responses may be a parasite strategy to promote the chronicity of infection (Recker et al., 2004). Although variation among *P. chabaudi* clones in innate immune response induction has been described (Long et al., 2006, 2008), and immunocompromised mice (lacking all T-cells or CD4+ T-helper cells) have been used to test whether the adaptive immune response influences competition between *P. chabaudi* clones (Barclay et al., 2008; Raberg et al., 2006), the potential for cross-reactive antibodies to mediate competition among a wide range of *P. chabaudi* clones has not been assessed.

In this study, we measured variation among nine clones in the induction of cytophilic antibodies, which exhibit a range of specificities and have great functional importance in the system: they block parasite invasion and development within RBC, bind infected RBC (Cavinato et al., 2001) to facilitate uptake and destruction by phagocytes (Mota et al., 1998), interfere with merozoite dispersal following RBC rupture (Bergmann-Leitner et al., 2009, 2006; Li et al., 2001), and are ultimately required for resolution of *P. chabaudi* infection (von der Weid et al., 1996). To study potential variation in polyclonal stimulation of B-cells by the malaria clones, we measured antibodies binding to the "exoantigen" Keyhole Limpet Haemocyanin, or KLH, a large and antigenically complex molecule (Harris and Markl, 1999) that the animals never experienced *in vivo* and is often used to quantify variation in antigen-independent humoral immune potency (e.g., (Star et al., 2007)). To study the induction of clone-transcending antibody, we measured binding of antibodies to two recombinant malaria antigens, Apical Membrane Antigen-1 (AMA-1) and Merozoite Surface Protein-1_{19} (MSP-1_{19}). These antigens are both malaria vaccine candidates (Anders et al., 1998; Burns et al., 2004; Crewther et al., 1996; Dodoo et al., 2008; Hensmann et al., 2004) that are known to be polymorphic in *P. chabaudi* (Cheesman et al., 2009; Crewther et al., 1996; McKean et al., 1993). We expected that these polymorphisms may directly predict the ability of antibodies induced by one clone to bind other clones. Together, our measurements of general immune potency and binding capacity for malaria antigens aid prediction of the mode and strength of immune-mediated competition among clones.

2. Materials and methods

2.1. Experimental infections

P. chabaudi clones were originally isolated from thicket rats (*Thamnomys rutilans*) and cloned by serial dilution and passage (Beale et al., 1978). These clones were stored as cryopreserved blood stabilates and passaged through donor mice prior to experimental infection. The clones we used vary in growth rate and virulence (Long et al., 2008; Mackinnon and Read, 1999) and are listed here in order of ascending maximum parasitaemia (percentage of RBCs infected) achieved in our experiments: AS_{11943}, CW_{175}, DK_{116}, DS_{1671}, AT_{53}, ER_{494}, AJ_{4787}, CR_{518}, AQ_{218}, (subscript denotes the point in the lineage from which each clone originates; hereafter clones are identified by their two-letter codes, AS, CW, etc.). All the clones used were *P. chabaudi chabaudi* except for DS and DK which belong to the subspecies *P. chabaudi adami*.

Female C57/BL6 mice (Harlan UK), 16–18 weeks of age, were housed in a 12 h:12 h light–dark cycle, and 41B diet (Harlan UK) and drinking water supplemented with 0.05% para-amino benzoic acid (PABA) were provided *ad libitum* (Jacobs, 1964). For each clone, we established infections by intraperitoneal injection of 1×10^5 *P. chabaudi* parasitised red blood cells (pRBCs). We used 5 experimental mice per clone, except for AS where 6 mice were used. Parasitaemia was monitored daily by $\times 1000$ microscopy of thin tail-blood smears stained with Giemsa, as described previously (Mackinnon and Read, 1999). Mice were exsanguinated, under terminal anaesthesia, in the early chronic phase of infection (day 35 post-infection). This timepoint was at least 3 weeks after the resolution of acute infection (the primary peak in parasitaemia) and is expected to reflect maximum antibody production (Quin and Langhorne, 2001), though measurable responses persist for over 6 months (Achtman et al., 2007). Serum was separated using SeraSieve (Hughes and Hughes Ltd) by centrifugation at 13,000 rpm for 10 min and stored at −80 °C. Protocols for this animal work were approved by the UK Home Office.

2.2. Antigens and antibody measurement

Three proteins were used in this study: Keyhole Limpet Haemocyanin, or KLH (SIGMA) and two recombinant malaria antigens; Merozoite Surface Protein-1_{19} (MSP-1_{19}) and Apical Membrane Antigen-1 (AMA-1). MSP-1_{19} was originally sequenced from *P. chabaudi chabaudi* clone AS and inserted into *Pichia pastoris* vector pIC9K for expression in *P. pastoris* strain SMD1169 as described previously (Hensmann et al., 2004). Apical Membrane Antigen-1 (AMA-1) was originally sequenced, cloned and expressed from *P. chabaudi adami* clone DK. The AMA-1 nucleotide sequence was inserted into *Escherichia coli* vector PQE9 and expressed in the cell line SG13009 (Crewther et al., 1996).

We used Enzyme Linked Immunosorbent Assays (ELISA) to measure antigen binding of serum immunoglobulin (Ig) isotypes IgG1, IgG2a, IgG2b and IgG3 as described previously (Fairlie-Clarke et al., 2010), with the following adjustments. Serum samples were added in a series of doubling dilutions (1/100 to 1/204800) using TBST as diluent. Antibodies that bound antigen were detected with isotype-specific hrp-conjugated goat anti-mouse antibodies (Southern Biotech: IgG1 1070-05 at 1/6000 dilution, IgG2a 1080-05 at 1/200, IgG2b 1090-05 at 1/4000 and IgG3 1100-05 at 1/1000). Antibody titres were calculated as the reciprocal of the greatest dilution at which the O.D. was greater than the mean plus 2 standard deviations of the O.D values for uninfected control samples binding that antigen at 1/100 dilution. Although all four isotypes were measured results are only presented for the most functionally relevant IgG2a.

2.3. Sequencing and bioinformatics

For each clone, when a suitable level of parasitaemia (minimum 5% parasitised RBCs) was observed in the donor mice, we collected 10 μl of whole blood from the tail in an excess of citrate saline (500 μl) and centrifuged at 13000 rpm for 5 min. The resulting pellet was stored at −80 °C. We extracted genomic DNA using the Instagene DNA preparation kit for whole blood (BIO-RAD Cat no. 7326211) according to the manufacturer's instructions. Polymerase Chain Reaction (PCR) was used to amplify fragments of *msp-1* and *ama-1*, as follows. 8 μl of 1/100 dilution of genomic DNA was used as a template in a 20 μl PCR reaction in combination with 0.1 mM final concentration of the forward and reverse primers. Primers were designed to amplify a 350 base pair fragment of *msp-1* and a 205 base pair fragment of *ama-1*. The region of MSP-1_{19} amplified for the 9 clones was aligned with the AS sequence for *msp-1_{19}*, GenBank accession number L22982.1