The generation and evaluation of two panels of epitope-matched mouse IgG1, IgG2a, IgG2b and IgG3 antibodies specific for Plasmodium falciparum and Plasmodium yoelii merozoite surface protein 1–19 (MSP1<sub>19</sub>)

Jaime R. Adame-Gallegos*, Jianguo Shi ¹, Richard S. McIntosh², Richard J. Pleass*

Lab C4, Institute of Genetics, Queen’s Medical Centre, University of Nottingham, Nottingham NG7 2UH, UK

1. Introduction

Amongst mouse IgG subclasses, IgG2a and IgG2b are considered to be the most potent activators, and dominate in successful passive transfer experiments in rodent malaria. To explore the mechanism(s) by which the different mouse IgG subclasses may mediate a protective effect, we generated mouse IgG1, IgG2a, IgG2b and IgG3 specific for the C-terminal 19-kDa region of Plasmodium falciparum merozoite surface protein 1 (PfMSP1<sub>19</sub>), and to the homologous antigen from Plasmodium yoelii (P. yoelii), both major targets of protective immune responses. This panel of eight IgGs bound antigen with an affinity comparable to that seen for their epitope-matched parental monoclonal antibodies (mAbs) from which they were derived, although for reasons of yield, we were only able to explore the function of mouse IgG1 recognizing PfMSP1<sub>19</sub> in detail, both in vitro and in vivo. Murine IgG1 was as effective as the parental human IgG from which it was derived in inducing NADPH-mediated oxidative bursts and degranulation from neutrophils. Despite showing efficacy in vitro functional assays with neutrophils, the mouse IgG1 failed to protect against parasite challenge in vivo. The lack of protection afforded by MSP1<sub>19</sub>-specific IgG1 against parasite challenge in wild type mice suggests that this Ab class does not play a major role in the control of infection with mouse malaria in the Plasmodium berghei transgenic model.

© 2012 Elsevier Inc. Open access under CC BY license.

* Corresponding authors. Present address: Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK. Fax: +44 01518230383 (R.J. Pleass).
E-mail addresses: sbxjra@nottingham.ac.uk (J.R. Adame-Gallegos), richard.pleass@liverpool.ac.uk (R.J. Pleass).

¹ Present address: University of Liverpool, School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK.
² Present address: University of Nottingham, Academic Clinical Oncology, City Hospital, Nottingham NG5 1PB, UK.

Received 13 August 2011
Accepted 2 February 2012
Available online 10 February 2012

Keywords:
Mouse IgG
IgG subclasses
Epitope matched antibodies
Malaria

ARTICLE INFO

Abstract

Murine immunoglobulin G (IgG) plays an important role in mediating protective immune responses to malaria. We still know relatively little about which IgG subclasses protect against this disease in mouse models, although IgG2a and IgG2b are considered to be the most potent and dominate in successful passive transfer experiments in rodent malaria. To explore the mechanism(s) by which the different mouse IgG subclasses may mediate a protective effect, we generated mouse IgG1, IgG2a, IgG2b and IgG3 specific for the C-terminal 19-kDa region of Plasmodium falciparum merozoite surface protein 1 (PfMSP1<sub>19</sub>) and to the homologous antigen from Plasmodium yoelii (P. yoelii), both major targets of protective immune responses. This panel of eight IgGs bound antigen with an affinity comparable to that seen for their epitope-matched parental monoclonal antibodies (mAbs) from which they were derived, although for reasons of yield, we were only able to explore the function of mouse IgG1 recognizing PfMSP1<sub>19</sub> in detail, both in vitro and in vivo. Murine IgG1 was as effective as the parental human IgG from which it was derived in inducing NADPH-mediated oxidative bursts and degranulation from neutrophils. Despite showing efficacy in vitro functional assays with neutrophils, the mouse IgG1 failed to protect against parasite challenge in vivo. The lack of protection afforded by MSP1<sub>19</sub>-specific IgG1 against parasite challenge in wild type mice suggests that this Ab class does not play a major role in the control of infection with mouse malaria in the Plasmodium berghei transgenic model.

© 2012 Elsevier Inc. Open access under CC BY license.
of recombinant mouse IgG1, IgG2a, IgG2b and IgG3 targeting identical epitopes on Plasmodium falciparum and P. yoelii MSP119 (Fig. 1). These were then used to investigate the anti-malarial properties of the mouse subclasses in vivo.

2. Materials and methods

2.1. Generation of PfMSP119-specific antibodies

2.1.1. Construction of pVL-C1-mouse kappa light chain

For all primer sequences, nucleotides in bold refer to restriction enzyme sites whereas an underline highlights stop codons. The plasmid pVL-C1-mouse kappa was generated by a three step PCR. Primer pairs VL-C1for (5’GGC GTG CAC TCC GAT ATT GTG ATG ACC CAG 3’) and k-joint-rev (5’ggg aag atg gat aca gtt ggt gca gca tca gtt c 3’) were used to amplify the VL by PCR from pVKExpress C1, whilst introducing an ApaI restriction site on the 5’ end. A second PCR with primer pairs k-joint-for (5’gaa ctg atg ctg cac caa ctg tat cca tct tcc c 3’) and MK-rev (5’gac tag tcc ctc taa cac tca ttc ctt gta 3’) containing a SpeI were used to amplify the mouse kappa light chain constant region from BALB/c genomic DNA. Overlap PCR with primer pairs VL-C1for and MK-rev were then used to fuse as a single PCR product the VL of C1 and the mouse kappa constant region, which was then cloned in pCR2.1-Topo (parking vector) prior to sub-cloning into the parental pVKExpress C1 (McIntosh et al., 2007; Persic et al., 1997) as an ApaI/SpeI fragment.

2.1.2. Construction of pVH-C1-mIgG1

pVH-C1-IgA1 (Shi et al., 2011) was digested with Sall to remove the IgA constant region sequence and religated to produce pVH-C1-delIgG1. This plasmid was then digested with BssHII/Xbol to release the VH of C1 which was then sub-cloned into pcDNA3.1-C1-hlgG1 from which the C1 and hlgG1 sequences were previously deleted (pcDNA3.1-delC1-delhlgG1) using the same restriction sites to create pcDNA3.1-C1-delhlgG1. The murine IgG1 constant region was amplified from BALB/c genomic DNA using primer pairs Mg1-for (containing an XbaI site) (5’GCA AAA GAG CGG CCT TCT AGA AGG TTT G 3’) and Mg1-rev (5’CAC TGG GAT CAT TTA CCA GGA GAG TG 3’) and cloned into the parking vector, prior to sub-cloning as an Xbol fragment into pcDNA3.1-C1-delhlgG1 to create pVH-C1-mlgG1.

2.1.3. Construction of pVH-C1-mlgG2a

Primer pairs Mg2a-for (5’GGTC ACC ATC AAG AGG AGG AAG 3’) and Mg2a-rev (5’TCT AGA GCT CA TTT TTA CCC GGA GT 3’) were used to amplify the murine IgG2a from genomic DNA whilst introducing a BstEII site 5’ of the CH1 and an XbaI site 3’ of the stop codon respectively and cloned into the parking vector. pVH-C1-hlgG1 was digested with Sall and blunted with T4 DNA polymerase (New England Biolabs) prior to recovery from gels, and digestion with Xbol, into which a BstEII blunted/XbaI fragment from the parking vector was ligated to create pVH-C1-mlgG2a.

2.1.4. Construction of pVH-C1-mlgG2b and IgG3

Primer pairs Mg2b-for (5’GATTG GCC CAT ACC GCA AGC TCT 3’) and Mg2b-rev (5’TCT AGA CCT CAT TTA CCC GGA GA 3’) were used to amplify the murine IgG2b from genomic DNA whilst introducing an EcoRV site 5’ of the CH1 and an Xbol site 3’ of the stop codon respectively and cloned into the parking vector. pVH-C1-hlgG1 was digested with BsmHII and blunted with T4 DNA polymerase (New England Biolabs) prior to recovery from gels, and digestion with Xbol, into which an EcoRV blunted/Xbol fragment from the parking vector was ligated to create pVH-C1-mlgG2b. Primer pairs Mg3-for (5’GATTG GTT CAG GAT AGA CCT GGG 3’) and Mg3-rev (5’TCT AGA CCT CAT TTA CCC GGA GA 3’) were used in the same way to generate pVH-C1-mlgG3.

![Fig. 1. Generating two panels of epitope-matched murine IgG Abs specific for MSP119 epitopes C1 (P. falciparum-PfMSP119) or B10 (P. yoelii-PyMSP119). Diagram represents a general overview to the construction of (a) C1 and (b) B10 mouse IgGs. Variable genes from parental mAbs C1 or B10 were sub-cloned into redesigned expression vectors for each of the mouse IgG subclasses.](image-url)
2.2. Generation of PyMSP119-specific antibodies

2.2.1. Construction of pVL-B10-mouse kappa light chain

pVL-C1-mouse kappa light chain was digested with ApaLI/XhoI to release the VL of C1. The VL of B10 was subcloned as an ApaLI/XhoI fragment derived from the parental expression vector pB10-VL/10 (Pleass et al., 2003).

2.2.2. Construction of pVH-B10-mlgG2a

pVH-C1-mlgG2a and parental plasmid pB10-VH/5 (Pleass et al., 2003) were digested with BssHII/BstEII to release the VH of C1 (pVH-delC1-mlgG2a) and the VH of B10 respectively. The VH of B10 was sub-cloned into pVH-delC1-mlgG2a using the same restriction sites and renamed pVH-B10-mlgG2a.

2.2.3. Construction of pVH-B10-mlgG1

The plasmid pVH-B10-mlgG1 was generated in two steps. pVH-C1-mlgG1 and pVH-B10-mlgG2a were digested with BssHII/BstEII to release the VH of C1 and VH of B10 respectively. The VH of B10 was sub-cloned into pVH-delC1-mlgG1. Due to the presence of a second BstEII site in exon 1 (CH1 domain) 160 bp after the VH of B10 3’ BstEII site, both plasmids were digested with BstEII and the released fragment sub-cloned into pVH-B10delC1-mlgG1 with the same restriction sites to generate pVH-B10-mlgG1.

2.2.4. Construction of pVH-B10-mlgG2b and IgG3

pVH-B10-mlgG2a was partially digested with MfeI/XhoI to release the IgG2a (pVH-B10-delmlgG2a) sequence after the VH of B10. IgG2b and IgG3 were released from their respective parking vectors with EcoRI/XhoI and sub-cloned into pVH-B10-delmlgG2a compatible cohesive restriction site ends (MfeI-EcoRI) and XhoI. The newly generated expression vectors were renamed as pVH-B10-delmlgG2b and pVH-B10-mlgG3. All plasmids were sequenced in both directions to confirm that no errors were introduced into either the variable or constant region genes during PCR/cloning.

Amino acid translation of the VH and VL genes for both C1 and B10 were expressed as follows: IgG1 on HEK-293T and IgG2a, IgG2b, and IgG3 on CHO-K1. Positive clones secreting MSP119-specific IgG detected by ELISA, on plates coated with recombinant PyMSP119-GST (Burghaus and Holder, 1994) or PyMSP119-GST (Lewis, 1989), or immuno-blotting using goat anti-mouse IgG-Fc (Pierce), goat anti-mouse IgG1, IgG2a, IgG2b or IgG3 (Southern Biotech), or a goat anti-mouse kappa (Southern Biotech) all conjugated to horseradish peroxidase (HRP) as previously described (Pleass et al., 2003). From large-scale cultures, mouse IgG was purified on HiTrap G-Sepharose (GE Healthcare) by FPLC. The integral and purity of the antibodies were verified on 4–12% SDS–PAGE gels (Invitrogen).

2.4. Immunofluorescence assay (IFA) and surface plasmon resonance (SPR) analysis

For IFAs, washed erythrocytes from mice infected with the transgenic (Tg) parasite Pb-PIM19 described by de Koning-Ward and colleagues (2003), were fixed on slides in methanol–acetone (1:1, vol/vol) for 10 min. After being blocked in phosphate buffered saline (PBS)/5% (vol/vol) goat serum, slides were incubated with Abs at 5 μg ml−1 in blocking buffer for 1 h, washed, then incubated for 1 h with fluorescein isothiocyanate (FITC)-conjugated goat F(ab’2) anti-human IgG (Sigma or Caltag) or goat anti-mouse IgG γ-chain-specific (Southern Biotech) diluted 1:500 in blocking buffer. After washing, slides were mounted with DAPI anti-fade and Ab binding examined by IF microscopy on a Zeiss AxioScope 40 microscope. For SPR, quantitative association (K_on) and dissociation (K_off) constants of the mouse IgG subclasses were measured using a BIAcore X Machine (GE Healthcare). Antigen (PyMSP119-GST) at a concentration of 50 μg/ml was amine-coupled onto a dextran matrix CMS sensor chip using a Pharmacia Biosensor Amine Coupling Kit following the manufacturer’s instructions. Various concentrations of purified IgGs were then injected into a final volume of 50 μL at a rate of 10 μL/min over the chip and binding kinetics analysed using BIAsimulation 3.0 software.

2.5. Luminol chemiluminescence assay of respiratory burst and myeloperoxidase release

Neutrophils were isolated from heparinized blood taken from healthy volunteers by the sedimentation of erythrocytes in 6%
(w/v) dextran T70 (GE Healthcare) in 0.9% (w/v) saline at 37 °C for 30 min, followed by leucocyte separation on a discontinuous density gradient of Lymphoprep ($\rho = 1.077$ g/cm$^3$; Nycomed, Birmingham, UK) over Ficoll-Hypaque ($\rho = 1.119$ g/cm$^3$), centrifuged at 700g for 20 min at room temperature. Approval for the collection and use of human cells was obtained from the local Queen's Medical Centre ethics committee. Wells of chemiluminescence microtiter plates (Dynatech Laboratories, Billinghurst, Sussex, UK) were coated with 150 $\mu$l of $\text{PfMSP1}_{19}$ at 5 $\mu$g ml$^{-1}$ in coating buffer (0.1 M carbonate buffer, pH 9.6) and incubated overnight at 4 °C. After washing three times with PBS, 150 $\mu$l of anti-$\text{PfMSP1}_{19}$ IgG at 100 or 50 $\mu$g ml$^{-1}$ was added to antigen coated wells. In each case, triplicate wells were prepared and left for 2 h at room temperature. After washing as before, 100 $\mu$l of luminol [67 $\mu$g ml$^{-1}$ in Hank's buffered saline solution (HBSS) containing 20 mM HEPES and 0.1 g/100 ml globulin-free BSA (HBSS/BSA)] was added to each well. After the addition of 50 $\mu$l of purified neutrophils (10$^9$/ml in HBSS/BSA) to each well, plates were transferred to a Microlumat LB96P luminometer, and the chemiluminescence was measured at 2 min intervals for 120 min at 37 °C. Data were analysed using Excel software.

2.6. Passive immunization and parasite challenge

Pathogen-free BALB/c mice from 6 to 8 weeks of age (caged individually) were used to test in vivo efficacy of mouse IgG1 in passive transfer experiments. IgG1 was administered (0.5 mg/injection) intraperitoneally (i.p.) on day −1, 0, and +1. Mice were challenged with 5000 parasitized red blood cells (prbc) with $\text{Pb-PfM19b}$ that were administered i.p. 5 h after Ab injection on day 0. From day +2 mice were screened daily for weight loss and % infected erythrocytes (parasitemia) counted by blood smears stained with Giemsa (Sigma). At the end of the experiment or when mice lost more than 20% of their initial weight, the animals were humanely sacrificed. All animal experiments were approved by the Home Office and performed in accordance with UK guidelines and regulations (PPL 40/2753).

3. Results

3.1. Characterization of $\text{PfMSP1}_{19}$-specific mouse IgG1, IgG2a, IgG2b and IgG3

$\text{PfMSP1}_{19}$ and $\text{PyMSP1}_{19}$-specific mouse IgG1, IgG2a, IgG2b and IgG3 were secreted into culture supernatants from stably transfected CHO-K1 or HEK-293T cells. These recombinant antibodies recognized either recombinant $\text{PfMSP1}_{19}$ or $\text{PyMSP1}_{19}$ by ELISA with polyclonal anti-mouse IgG Fc- or kappa light chain-specific secondary reagents (Fig. 3a and b). To check that each cell line only secreted the intended antibody class, a second sandwich ELISA was developed to confirm the subclass of the secreted IgG antibodies (Fig. 3c and d). Here we focus on the $\text{PfMSP1}_{19}$-specific mouse IgG1, IgG2a, IgG2b and IgG3 that were purified from CHO-K1 or HEK-293T culture supernatants by protein-G affinity-chromatography and appeared pure by SDS–PAGE analysis (Fig. 4a). Total yields of protein after purification, dialysis, and concentration from numerous different batches of culture supernatant over several time points were in the region of 15 mg from 30 l of supernatant for IgG1, and 2.8 mg, 2.1 mg, 1.5 mg from 7 l of supernatant for IgG2a, IgG2b and IgG3.

![Fig. 3. ELISA screening of PfMSP1$_{19}$ and PyMSP1$_{19}$-specific mouse IgGs. HEK-293T or CHO-K1 mammalian cell lines were stably-transfected by electroporation with expression plasmids coding for the panel of mouse IgGs targeting P. falciparum or P. yoelii MSP1$_{19}$. Clones secreting antigen-specific IgGs were detected by enzyme-linked immunosorbent assay (ELISA). Five micrograms of recombinant purified antigen PfMSP1$_{19}$ (a) or PyMSP1$_{19}$ (b) was coated and secreting clones detected with an anti-mouse IgG Fc-specific (HC) or anti-mouse kappa light chain (LC) secondary antibodies. To confirm the secretion of the panel of C1 (c) or B10 (d) epitope-matched mouse IgGs a capture ELISA was performed. Plates were coated with goat anti-mouse F(ab)2 and supernatant from secreting clones analysed with subclass-specific secondary antibodies. All secondary reagents used were HRP conjugated and all plates were read at 450 nm. Bars represent the mean OD values from duplicate or triplicate wells. Supernatant from untransfected cells was used as negative control. Two micrograms of mAb B10 was used as positive control on panel b. Error bars represent Standard Deviation (SD).](image-url)
respectively. The poor yields of IgG2a, IgG2b and IgG3 were most likely a consequence of these subclasses being cloned into a different expression plasmid (pVHExpress) compared to the mouse IgG1 (pCDNA3.1), and therefore the majority of work described focuses on IgG1 for which a sufficient quantity of antibody was derived for in vivo studies. The mouse IgG antibodies had an apparent molecular weight (MW) of approximately 150 kDa, although there were minor differences in the MW dependent on subclass. All the preparations contained a significant proportion of free heavy and light chains (arrowed), suggesting that not all the expressed protein folded into intact heterodimeric antibody. Western blotting with the IgG subclass-specific reagents used in the detecting ELISAs were unable to detect any of the mouse IgGs by immunoblotting on both non-reducing and reducing gels, although an alternative polyclonal goat anti-mouse IgG-Fc did recognize mouse IgG1, IgG2a and very faintly IgG2b (Fig. 4b). No significant binding to mouse IgG3 was observed with this reagent despite the presence of an equivalent amount of protein. Importantly, surface plasmon resonance (SPR) analysis for all the IgG subclasses generated revealed no lent amount of protein. Importantly, surface plasmon resonance was observed with this reagent despite the presence of an equivalent amount of protein. Importantly, surface plasmon resonance (SPR) analysis for all the IgG subclasses generated revealed no reduction in affinity for P.falciparum MSP119 when compared with the parental human IgG1 antibody (Table 1 and Fig. 5), the binding constants remaining essentially the same for each subclass. The recombinant mouse IgG1 by indirect IFA produced a characteristic pattern of MSP1 reactivity from P. falciparum infected erythrocytes (not shown) and Plasmodium berghei parasites Tg for PfMSP119 (Fig. 6, bottom right corner, arrowed).

Table 1

| Antibody | $K_a$ (1/M) | $K_d$ (M) |
|----------|------------|----------|
| mlgG1-C1 | $4.48 \times 10^7$ | $2.3 \times 10^{-8}$ |
| mlgG2a-C1 | $3.32 \times 10^7$ | $3.01 \times 10^{-8}$ |
| mlgG2b-C1 | $1.2 \times 10^7$ | $8.41 \times 10^{-8}$ |
| mlgG3-C1 | $2.33 \times 10^7$ | $3.11 \times 10^{-8}$ |
| lgG1-C1 | $3.94 \times 10^7$ | $2.3 \times 10^{-8}$ |

* Methodology described in McIntosh et al. (2007) and Pleass et al. (2003).

### 3.2. IgG1 triggers PfMSP119-specific neutrophil nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation through FcγR cross-linking

We next assessed the ability of the mouse IgG1 to interact with FcγRs and induce NADPH oxidase activation (respiratory burst) and degranulation in blood neutrophils (Fig. 7). For neutrophils, luminol chemiluminescence provides a read out of NADPH oxidase activation and myeloperoxidase release (Pleass et al., 2007, 2003). When attached to PfMSP119-GST-coated plates, the mouse IgG1-induced comparable respiratory bursts to the parental human IgG1 (Fig. 7), suggesting that binding to PfMSP119 allows mouse IgG1 to be presented to neutrophil FcγRs in an optimal configuration for receptor cross-linking and triggering of functional responses. This finding is similar to that reported previously with a different mouse IgG1 (mAb 12.10) also recognizing PfMSP119 (Lazarou et al., 2009). Since mouse IgG1 is known to bind to human FcγRIIA but not to FcγRIII, the activation of these neutrophils most likely occurs through binding to the former receptor (Pleass et al., 2003).

### 3.3. Passive transfer of mouse IgG1 into wild type mice has no effect on the course of a rodent malaria infection

Despite binding PfMSP119 with high affinity, passive transfer of mouse IgG1 into WT BALB/c mice failed to protect seven out of eight animals, in two separate experiments, from a challenge infection with blood-stage P. berghei parasites Tg for PfMSP119 (Fig. 8).

### 4. Discussion

Although we have previously shown that mice Tg for human FcRs are very useful for investigating human antibody function in vivo, particularly where there are no suitable animal models for P. falciparum infection in humans (McIntosh et al., 2007; Shi et al., 2011), an argument can be made that they are somewhat contrived, and therefore should be compared in parallel experiments in wild type models of murine malaria infection with murine antibodies. Here we describe the construction of two panels of mouse IgG1, IgG2a, IgG2b and IgG3 recognizing identical epitopes in PfMSP119 or PyMSP119.

Plasmodium spp. merozoites attach to the surface of red blood cells by means of different surface antigens; among these antigens is MSP1 (Holder, 2009). This surface antigen is cleaved twice; the first cleavage takes place at schizonts followed by a second cleavage to reveal PfMSP119 just prior to invasion (Blackman et al., 1994; Morgan et al., 1999). This 19-kDa peptide is relatively conserved with few polymorphic variants and has been described as a major candidate for vaccine development (Pizarro et al., 2003), and continues to be the subject of study for immune therapy (Roussillon et al., 2007).

These epitope-matched antibodies were generated by cloning variable genes from mAbs previously shown to confer passive protection from rodent malaria (McIntosh et al., 2007; Spencer Valero et al., 1998), into newly generated expression constructs containing mouse IgG1, IgG2a, IgG2b and IgG3 constant region genes.
respectively. The original plan was to generate the panel of mouse IgGs with the same expression plasmid (pcDNA3.1). However, due to the presence of a BstEII site on the C-terminal site of the VH-C1 in the parental human expression plasmid, we could not find a suitable enzyme site with which releases the human IgG1 sequence from the expression vector and subclones all the mouse IgG sequences in. The approach taken to generate the IgG2a, IgG2b, and IgG3-C1 Abs was to sub-clone in the murine sequences in an

![Surface plasmon resonance (SPR)-derived association and dissociation curves of mouse IgGs binding to PfMSP119.](image1)

**Fig. 5.** Surface plasmon resonance (SPR)-derived association and dissociation curves of mouse IgGs binding to PfMSP119. SPR association ($K_A$) and dissociation ($K_D$) curves of mouse IgG binding to recombinant purified antigen PfMSP119 amine-coupled and immobilized to a CM5 sensor chip. The antibodies were injected into flow at five different concentrations of 2 μM (red), 1 μM (green), 0.5 μM (cyan), 0.25 μM (pink), and 0.125 μM (dark blue) at time 0 and replaced with buffer alone at the times indicated with arrows. No binding was seen from any of the IgGs onto control Ag coated in flow cell 2. Data are summarized in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

![Characterization of purified mouse IgG1-C1 by immunofluorescence assay (IFA).](image2)

**Fig. 6.** Characterization of purified mouse IgG1-C1 by immunofluorescence assay (IFA). Mouse IgG1 is reactive with MSP119 on methanol–acetone-fixed smears of merozoites and erythrocytes infected with rodent *P. berghei* Tg for *P. falciparum* MSP119 (Pf-PfM19). Top panel: mAbs 12.8 and 12.10 previously described and known not to bind to Pf-PfM19 (Lazarou et al., 2009) and B10 anti-µMSP119 as negative control (Spencer Valero et al., 1998). Bottom panel: Human IgG1-C1 (JS1) and human IgG1-e9 (JS2) as previously described (McIntosh et al., 2007) and murine IgG1-C1 bind Pf-PfM19 infected erythrocytes. Binding was visualized by staining with a FITC-conjugated secondary reagent with nuclei stained with DAPI and assessed by fluorescent microscopy under 40× magnification.
expression plasmid (pVHExpress) previously used in the expression of anti-malarial Abs (McIntosh et al., 2007). These plasmids were expressed in CHO-K1 or HEK-293T cells, and all eight secreted antibodies generated were shown to bind their respective MSP119 antigen, and were identified as belonging to the correct subclass by ELISA (Fig. 2).

CHO and HEK-293 mammalian cell lines are widely used in the production of recombinant proteins (Wurm, 2004). Predominantly in industry, CHOs have been used to express a wide range of recombinant proteins including antibodies used in the treatment of numerous diseases, including: multiple sclerosis, arthritis and cancer (Hossler et al., 2009; Kelley, 2009). To a lesser extent,
HEK-293s have also been used in the industry to produce recombinant antibodies, but alongside CHOs, they have been the subject of study for increased productivity in the field (Wurm, 2004). HEK-293s have been used to express recombinant proteins for studies in neurology, electrophysiology and to produce mAbs amongst other therapeutic proteins (Chen et al., 2011; Thomas and Smart, 2005). Functional IgGs need to be glycosylated in their Fc-domain in order to have Fc-mediated effector functions (Jeffersis, 2005; Walsh and Jeffersis, 2006), although we have not directly assessed the glycosylation status of these reagents, CHO and HEK-293 cell lines have been previously shown to effectively secrete Abs with N-linked carbohydrates (Schlenke et al., 2007; Sethuraman and Stadheim, 2006).

The mouse IgG1, IgG2a, IgG2b and IgG3 all bound PfMSP1<sub>19</sub> with high affinity as determined by surface plasmon resonance analysis (Fig. 4). The panel of IgG antibodies bound with comparable affinity to recombinant PfMSP1<sub>19</sub> in line with previous observations with other antibodies (McIntosh et al., 2007), and the mouse IgG1 bound to native protein as determined by immunofluorescence microscopy with infected erythrocytes (Fig. 6). Previous results have shown that the parental human IgG1 from which these mouse antibodies were derived is dependent on FcR-mediated recruitment for function, and that inhibition of erythrocyte invasion and/or inhibition of MSP1 processing are/is not the principal mechanism through which this antibody controls parasites (McIntosh et al., 2007).

Typically in passive transfer experiments with rodent malaria, large concentrations of antibody (1.5 mg total dose per mouse, given as three individual doses) have to be given to provide protection. Although we could produce sufficient quantities of all the antibody classes to allow for preliminary in vitro comparisons to be made, we could only generate sufficient mlgG1 with which to do in vivo work. Intriguingly, passive transfer experiments with this IgG1 antibody failed to protect BALB/c mice with challenge infection with malaria parasites (Fig. 8). Although the mouse IgG1 was fully functional, as determined by its ability to induce NAPDH mediated oxidative bursts from human neutrophils, the antibody was ineffective at controlling the development of the malaria when passively transferred into BALB/c mice that were challenged with the Tg parasite Pb-PM19 (de Koning-Ward et al., 2003). In contrast to mouse IgG2a or IgG2b, mouse IgG1 binds preferentially to inhibitory FcγRIIB (CD32B) expressed on murine macrophages, monocytes and B cells (Nimmerjahn and Ravetch, 2006; Pleass and Woof, 2001), but not on neutrophils, offering a possible explanation as to why this subclass was found to be ineffective at controlling the development of a lethal malaria infection when passively transferred into recipient mice.

The similarities of the genomes of the human and rodent Plasmodium spp. with their 14 chromosomes, ranging from 23 to 27 Mb, coding for over 5000 proteins from which around 20% of the +5300 genes found in these genomes are orthologous, highlight the importance of murine malaria as models to understand the human disease (Doolan, 2011; Hall et al., 2005; Janse et al., 2010). These genetic similarities have led to the development of targeted gene knock-outs, mutations, fluorescently-tagged, generation of ‘reporter’ or ‘attenuated’ parasites that contribute to the development of reliable vaccines against the disease (Janse et al., 2010). To date, 561 rodent parasite mutants (predominantly P. berghei) have been reported (Rodent Malaria genetically modified Parasites DataBase (RMgmDB) http://www.pberghei.eu/ accessed on 11th August 2011).

The use of Tg P. berghei rodent parasites expressing vaccine-candidate Ags from orthologous genes of P. falciparum has been described for a wide range of proteins and genes, such as the: sporozoite adhesive protein TRAP (Wengelnik et al., 1999), circumsporozoite protein (CSP) (Persson et al., 2002; Tewari et al., 2002), the chloroquine resistance transporter (CRT) (Ecker et al., 2011), and the UIS4 and HT1 genes that code for a parasitophorous vacuole membrane protein in sporozoites and liver stages (expressed in a Tg P. yoelii) and a hexose transporter, respectively (Blume et al., 2011; Mackellar et al., 2010).

Parasites Tg for MSP1<sub>19</sub> are no exception. Rodent parasite Plasmodium chabaudi MSP1<sub>19</sub> has been expressed in P. falciparum and used for in vitro studies (Corran et al., 2004), and two Tg rodent P. berghei parasites expressing PfMSP1<sub>19</sub> have been described: Pb-PM19 and PMSP1-19H<sub>b</sub> (Cao et al., 2009; de Koning-Ward et al., 2003) (respectively). Passive immunization of human IgG1 anti-PfMSP1<sub>19</sub> in BALB/c mice challenged and infected with Pb-PM19, protected CD64-Tg mice (but not WT mice) from the course of infection (McIntosh et al., 2007). Similar results were found in mice immunized with purified rabbit anti-PfMSP1<sub>142</sub> IgG where sterile protection was also observed (Sachdeva et al., 2006). However, alternatively from these experiments, passive transfer of Abs 12.8 and 12.10 into BALB/c mice challenged with Pb-PM19 confirmed their inability to protect in vivo suggesting that the presence of six amino acids of P. berghei’s MSP1<sub>19</sub> in the Tg parasite was required for both mAbs to be able to bind to the PfMSP1<sub>19</sub> epitope (Lazarou et al., 2009). These discrepant results from passive transfer experiments in infections with Pb-PM19 have encouraged us to assess the efficacy of the alternate panel of IgGs targeting PfMSP1<sub>19</sub> to elucidate their response in a more natural model of infection.

From 1965 to 2010, more than 1900 immunization studies have been conducted involving all four rodent Plasmodium spp. and human parasites in feasible vaccine development trials. In this vast amount of time and research, many combinations of treatments have been studied such as: (1) immunizations with live, attenuated or dead parasites, (2) purified proteins such as Ags, Abs, DNA subunits, via multiple routes of delivery (Guilbride et al., 2010). Passive transfer experiments in vivo looking at the efficacy of Abs in controlling malaria continue to be of interest as still, the mechanisms of action of the Fc-portion of Abs in controlling the disease is not completely understood. Current improvements in the mass production of recombinant proteins could allow IgG therapies to become the standard choice of treatment against blood-stage malaria in the near future. It is of our interest to continue this project and aim to increase the yield of purified Abs from transfected mammalian cell lines by expressing our panel of IgGs in Sp2/0 myelomas for instance. With greater yields of purified Abs, we would be able to complete the in vitro characterization of the remaining -C1 Abs and compare in vivo both panels of the epitope-matched IgGs constructed.

In summary, two panels of epitope-matched mouse IgGs (IgG1, IgG2a, IgG2b, and IgG3) targeting PfMSP1<sub>19</sub> or PyMSP1<sub>19</sub> were constructed and expressed in mammalian cell lines. A novel PfMSP1<sub>19</sub>-specific mouse IgG1 did not show protective capability against parasite challenge in mice but was shown to be fully functional in vitro. Whilst this finding may indicate that IgG1 does not play a major role in protection against malaria, we cannot rule out the possibility that the findings reflect certain shortcomings of the PfMSP1<sub>19</sub> Tg P. berghei rodent malaria model used, hence future work aims to investigate the role of the other IgG subclasses generated using the anti-P. yoelii panel of antibodies described herein.

Acknowledgments

We thank the Wellcome Trust for provision of funding for this work (082915/B/07/Z) and the Mexican Council of Science and Technology (CONACYT) for provision of a PhD studentship to J.A.-G. We thank Tania de Koning-Ward and Brendan Crabbe (WEHI, Melbourne) for provision of the P. berghei transgenic parasites, and
Tony Holder (NIMR) for the original B10 hybridomas and provision of recombinant MSP119.

References

Blackman, M.J., Scott-Finnigan, T.J., Shai, S., Holder, A.A., 1994. Antibodies inhibit the protease-mediated processing of a malaria merozoite surface protein. J. Exp. Med. 180, 389–393.

Blume, M., Hliscs, M., Rodriguez-Contreras, D., Sanchez, M., Landfear, S., Lucius, R., Matuschewski, K., Gupta, N., 2011. A constitutive pan-hemocyte response for the Plasmodium life cycle and transgenic models for screening of antimalarial sugar analogs. FASEB J. 25, 1218–1229.

Burghaus, P.A., Holder, A.A., 1994. Expression of the 19-kilodalton carboxy-terminal fragment of the Plasmodium falciparum merozoite surface protein-1 in Escherichia coli as a coiled-coiled folded protein. Mol. Biochem. Parasitol. 64, 165–169.

Cao, V., Zhang, D., Pan, W., 2009. Construction of transgenic Plasmodium berghei as a model for evaluation of blood-stage vaccine candidate of Plasmodium falciparum chimeric protein 2.9. PLoS One 4, e6894.

Chen, Z., Chumakov, K., Dragunski, E., Kovaliavka, D., Makiya, M., Neverov, A., Reznykh, G., Sobell, A., Purcell, R., 2011. Chimpanzee/human monoclonal antibodies for treatment of chronic poliovirus exacerbations and emergency post-exposure prophylaxis. J. Virol. 85, 4354–4362.

Chu, N., Thomas, B.N., Patel, S.R., Buxbaum, L.U., 2010. IgG is pathogenic in Leishmania mexicana infection. J. Immunol. 185, 6509–6546.

Clynes, R.A., Towers, T.L., Presta, L.G., Ravetch, J.V., 2000. Inhibitory Fc receptors mediate in vivo cytotoxicity against tumor targets. Nat. Med. 6, 443–446.

Coran, P.H., O’Donnell, R.A., Todt, J., Uthaitiphaib, C., Holder, A.A., Crab, B.S., Riley, E.M., 2004. The fine specificity, but not the invasion inhibitory activity, of 19-kilodalton merozoite surface protein 1-specific antibodies is associated with resistance to malaria parasitemia in a cross-sectional survey in the Gambia. Infect. Immun. 72, 6185–6189.

de Koning-Ward, T.F., O’Donnell, R.A., Drew, D.R., Thomson, R., Speed, T.P., Crab, B.S., 2003. A new rodent model to assess blood stage immunity to the Plasmodium falciparum antigen merozoite surface protein 19 reveals a protective role for antibodies elicited in the liver. J. Exp. Med. 198, 869–875.

Doolan, D.L., 2011. Plasmodium immunomics. Int. J. Parasitol. 41, 3–20.

Ecker, A., Lakshmanan, V., Sinnis, P., Coppsen, I., Fidock, D.A., 2011. Evidence that MSP1-19, the C-terminal fragment of merozoite surface protein-1 from Plasmodium falciparum: a malaria vaccine candidate. J. Mol. Biol. 328, 1091–1103.

Pleass, R.J., Lang, M.L., Kerr, M.A., Wood, J.M., 2007. IgA is a more potent inducer of NADPH oxidase activation and degranulation in blood eosinophils than IgM. J. Immunol. 177, 1401–1408.

Pleass, R.J., Ogun, S.A., McGuinness, D.H., van de Winkel, J.G., Holder, A.A., Wood, J.M., 2003. Novel antimalarial antibodies highlight the importance of the antibody Fc region in mediating protection. Blood 102, 4424–4430.

Pleass, R.J., Wood, J.M., 2001. Fc receptors and immunity to parasites. Trends Parasitol. 17, 545–551.

Reitan, S.K., Hannestad, K., 1995. A syngeneic idiotype is immunogenic when both by IgM but tolerogenic when joined to IgG. Eur. J. Immunol. 25, 1601–1608.

Reitan, S.K., Hannestad, K., 2002. Plasmodium falciparum heavy chain constant regions regulate immunity and tolerance to idiotypes of antibody variable regions. Proc. Natl. Acad. Sci. USA 99, 7588–7593.

Rodrigo, W.W., Block, O.K., Lane, C., Sukupolvi-Petty, S., Gonzalez, A.P., Johnson, S., Diamond, M.S., Lai, C.J., Rose, R.C., Jin, X., Schlesinger, J.J., 2009. Dengue virus neutralization is modulated by IgA antibody subclass and Fc gamma receptor usage. J. Virol. 83, 3475–3487.

Roussillon, C., Ouvry, C., Muller-Graf, C., Tall, A., Rogier, C., Trape, J.F., Matuschewski, K., Gupta, N., 2011. A constitutive pan-hexose permease for the infection. J. Immunol. 185, 6939–6946.

Sachdeva, S., Mohmmed, A., Dasaradhi, P.V., Crabb, B.S., Katyal, A., Malhotra, P., Rodrigo, W.W., Block, O.K., Lane, C., Sukupolvi-Petty, S., Goncalvez, A.P., Johnson, S., Diamond, M.S., Lai, C.J., Rose, R.C., Jin, X., Schlesinger, J.J., 2009. Dengue virus neutralization is modulated by IgA antibody subclass and Fc gamma receptor usage. J. Virol. 83, 3475–3487.

Spencer Valero, L.M., Ogun, S.A., Fleck, S.L., Ling, I.T., Scott-Finnigan, T.J., Blackman, M.J., Holder, A.A., 1998. Passive immunization with antibodies against three distinct epitopes on Plasmodium yoelii merozoite surface protein 1 suppresses parasitemia. Infect. Immun. 66, 3925–3930.

Tewari, R., Spaccapelo, R., Bistoni, F., Holder, A.A., Crisanti, A., 2002. Function of region 1 and 2 adhesive motifs of Plasmodium falciparum circumsporozoite protein in sporozoite motility and infectivity. J. Biol. Chem. 277, 47613–47618.

Therien, R., Smart, T.G., 2005. HEK293 cell line: a vehicle for the expression of recombinant proteins. J. Pharmacol. Toxicol. Methods 51, 187–200.

Torres, M., Fernandez-Fuentes, N., Fiser, A., Casadevall, A., 2007. The immunoglobulin heavy chain constant region affects kinetics and thermodynamic parameters of antibody variable region interactions with antigen. J. Biol. Chem. 282, 13917–13927.

Wald, G., Jeffers, R., 2006. Post-translational modifications in the context of therapeutic proteins. Nat. Biotechnol. 24, 1241–1252.

Wengelnik, K., Spaccapelo, R., Naitza, S., Robson, K.J., Janse, C.J., Bistoni, F., Waters, A.P., Crisanti, A., 1999. Aα-domain and the thrombospondin-related motif of
Plasmodium falciparum TRAP are implicated in the invasion process of mosquito salivary glands. EMBO J. 18, 5195–5204.
Wojciechowski, W., Harris, D.P., Sprague, F., Mousseau, B., Makris, M., Kusser, K., Honjo, T., Mohri, K., Mohr, M., Randall, T., Lund, F.E., 2009. Cytokine-producing effector B cells regulate type 2 immunity to H. polygyrus. Immunity 30, 421–433.
Woof, J.M., 2005. Immunology. Tipping the scales toward more effective antibodies. Science 310, 1442–1443.
Wurm, F.M., 2004. Production of recombinant protein therapeutics in cultivated mammalian cells. Nat. Biotechnol. 22, 1393–1398.