Protective immunity to erythrocytic *Plasmodium chabaudi* AS infection involves IFNγ-mediated responses and a cellular infiltrate to the liver

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SUMMARY

IFNγ receptor (IFNγR) deficient mice and IL-4 deficient mice were infected with blood-stage *Plasmodium chabaudi* AS in order to analyse the role of Th1 (IFNγ) and Th2 (IL-4)-associated cytokines in the development of protective immunity to the parasite. A high mortality rate and failure to reduce the primary parasitaemia to subpatent levels was observed in the IFNγR deficient mice. IL-4 deficient mice controlled a primary *P. chabaudi* AS infection in a similar manner to control mice and no mortality was observed. IFNγR deficient mice had a reduction in parasite-specific IgG and a significantly increased level of total IgE compared to control mice. There was no reduction in the level of parasite-specific IgG in IL-4 deficient mice. Cytological analysis of the cells present in the spleen and liver during the primary parasitaemia revealed a reduction in the numbers of lymphocytes, monocytes and polymorphonuclear (PMN) cells in the liver at the peak of parasitaemia in both IFNγR deficient mice and IL-4 deficient mice compared to control mice. Adoptive transfer studies demonstrated that cells isolated from the liver at day 11 post-infection could confer some protective immunity to *P. chabaudi* AS infection.

Key words: IFNγ, IL-4, *Plasmodium chabaudi*, lymphomyeloid cells, liver.

INTRODUCTION

Regulation of the immune response by $CD4^+$ T helper (Th) cells and their cytokine products to an infection, can determine the outcome of that infection. For example, resistance to *Leishmania major* infection in mice is associated with Th1/IFNγ-mediated responses whereas susceptible mice produce IL-4 and have a Th2 bias (Reiner & Locksley, 1995). *Plasmodium chabaudi* AS infection in resistant mice induces sequential involvement of Th1 and Th2 regulated responses (Langhorne *et al*. 1989). An increase in IFNγ production is observed during the acute primary parasitaemia of a *P. chabaudi* infection (Meding *et al*. 1990) and is thought to be essential for IL-12 and NO dependent protection (Stevenson *et al*. 1995). The Th2-dependent control of *P. chabaudi* infection immediately following acute primary parasitaemia is thought to involve IL-4 and antibody-dependent mechanisms (Taylor-Robinson & Phillips, 1994).

The development of cytokine and cytokine receptor deficient mice has allowed the investigation of individual cytokines in protective immune responses to infection. We have used IFNγR deficient mice and IL-4 deficient mice to analyse the role each of these cytokines has during a *P. chabaudi* blood-stage infection, and to allow a degree of comparison between Th1 and Th2 mediated responses.

The results reported in this paper confirm and extend previous observations (Favre *et al*. 1997; Van Der Heyde *et al*. 1997) in that IFNγ-mediated responses are crucial for the development of protective immunity to a primary *P. chabaudi* infection whereas the absence of IL-4 did not alter the outcome of infection significantly. Cytological analysis of the cells present in the livers of both IFNγR deficient mice and IL-4 deficient mice revealed a reduction in the numbers of lymphocytes, monocytes and PMN cells compared to control mice. Subsequent experiments in the experimental model established in our laboratory (*P. chabaudi* infection of NIH mice) revealed an increase in lymphomyeloid cells present in the liver at peak parasitaemia. Adoptive transfer studies demonstrated that these lymphomyeloid cells were able to mediate protection to a *P. chabaudi* infection in an irradiated naïve recipient. The detailed analysis of the course of the parasitaemia in IFNγR deficient mice and IL-4 deficient mice together with analysis of the cellular infiltrates in the spleen and liver during *P. chabaudi* infection reported in this paper are novel data which

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extend the previous observations of Favre *et al.* (1997) and Von Der Weid *et al.* (1994). The demonstration that lymphomyeloid cells present in the liver during *P. chabaudi* infection can mediate protection suggests that the liver may be an important site of a protective immune response to a blood-stage *P. chabaudi* infection.

MATERIALS AND METHODS

Mice

Male IFNγR deficient mice (129SV × C57BL/6)F_2 and IL-4 deficient mice (129SV × C57BL/6)F_2 were generated as described (Kopf *et al.* 1993; Huang *et al.* 1993). The original breeding pairs were obtained by J. Alexander from H. Bluethmann, Basel. The mice were bred and maintained in the animal facility at the University of Strathclyde, Glasgow. For experiments other than those with IFNγR deficient mice and IL-4 deficient mice, inbred female NIH mice were used routinely for experimental infections. These mice were purchased from Harlan Olac (Bicester, UK) and kept in the University of Glasgow at the Joint Animal Facility. All mice used for experimental purposes were aged 6–12 weeks and weighed approximately 25 g.

Parasites

P. chabaudi chabaudi AS blood-stage parasites were isolated from adult thicket rats, established in laboratory mice and cloned by limiting dilution (Walliker *et al.* 1971). Stabilates of parasites, derived from the original AS parent parasite clone, were maintained by cryopreservation and subpassage through mice (Gray & Phillips, 1981).

Challenge infections

Parasites from frozen stock were injected i.p. into 2 passage mice and the parasitaemia was monitored daily by examination of Giemsa's-stained thin blood smears. When patent parasitaemia developed, infected blood was recovered in heparin (10 i.u./ml), diluted in RPMI 1640 medium to the required concentration (either 1×10^5 or 2×10^6 in 0·2 ml) of parasitized erythrocytes (pRBCs). Experimental mice were infected with pRBC administered i.v. as a 0·2 ml inoculum. Experimental groups consisted of 5 or 6 mice and the parasitaemias were monitored daily.

Presentation of parasitaemic data

The course of infection of a group of mice was represented by plotting the geometric mean of the parasitaemia (mean $\log_{10}$ of the number of pRBC in 10^5 RBC) or the mean percentage parasitaemia against time. For clarity's sake the standard deviation for each data-point has been omitted but significant differences at specific time-points are noted in the text.

Analysis of anti-malarial antibody production during infection

The slide IFAT procedure of Van Meirvenne, Janssens & Magnus (1975) modified by McLean, Pearson & Phillips (1982) was used to determine total anti-malarial antibody levels in the serum of infected mice. The procedure is an adaptation of the indirect fluorescent antibody method described by Voller (1964) and O'Neill & Johnson (1970) and has been described elsewhere (Taylor-Robinson & Phillips, 1992). Sera from 3 mice were harvested and the level of parasite-specific IgG was determined by indirect fluorescence. Results are the mean of 3 samples and are expressed as the reciprocal of antibody titre.

Determination of total IgG1 and IgG2a production

Sera were collected from mice and assayed for production of total IgG1 or IgG2a antibodies. Capture anti-antibody monoclonals were diluted to the required concentration (anti-IgG1 at 4 μg/ml, anti-IgG2a and anti-IgE at 2 μg/ml) in coating buffer (bicarbonate buffer, pH 8·2). Then 50 μl of capture antibody were aliquoted per well on an Immunlon 4 ELISA plate (Dynatech) and incubated overnight at 4 °C. The plate was washed twice with PBS/Tween (0·05 %). For each wash, wells were filled with PBS/Tween and allowed to stand for at least 1 min. The PBS/Tween was discarded and the plate was pounded onto paper towels after the final wash. The wells were then blocked by the addition of 200 μl/well of 10 % FCS/PBS for 1 h at 37 °C and the plate was subsequently washed twice as described above. Standards and samples were subsequently added at 50 μl/well at the required concentration. Standard IgG1 was added at 1 μg/ml, IgG2a at 0·4 μg/ml and IgE at 1 μg/ml. The serum samples were diluted 1:100 for both IgG1 and IgE assays and 1:500 for the IgG2a assay. The standards and samples were diluted in 10 % FCS/PBS. The plate was washed 4 times as before. Detecting monoclonal antibodies were diluted in 10 % FCS/PBS to the required concentration. Biotinylated anti-IgG2a and anti-IgE was used at 2 μg/ml. Biotinylated anti-IgG1 κ and λ chains were added in a 1:1 mixture at 2 μg/ml each and 50 μl of the respective detecting antibody were added per well and incubated at 37 °C for 1 h. The plate was washed 6 times in PBS/Tween and 100 μl of streptavidin–peroxidase (Sigma) at 2 μg/ml were added per well. The plate was incubated at 37 °C for 1 h, washed 8 times as before and then 100 μl/well of 3,3′,5,5′-tetramethyl-

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benzidine (TMB) substrate (KPL) was added and colour (blue) was allowed to develop (5–30 min). The plate was read at 630 nm with a reference filter at 405 nm on a MRX plate reader (Dynatech). The results of the unknown samples were calculated against a standard curve of known concentrations plotted using Biolinx software (Dynatech).

Preparation of cells from spleen, liver and peripheral blood

Spleens were removed aseptically, disrupted mechanically and the cells were washed in incomplete RPMI-1640 medium. Contaminating erythrocytes were lysed by resuspension of cells in 0·83% Tris–ammonium chloride (Tris–NH_4Cl, pH 7·4) for 5 min at room temperature. The spleen cell suspension was washed twice with 10% FCS–RPMI-1640 medium at 250 ***g*** for 5 min. The pellet was resuspended in 10% FCS–RPMI medium for determination of cell viability and number. Livers were excised aseptically from mice, dissected into small pieces and digested in a warmed (37 °C) solution of RPMI-1640 medium containing 50 U/ml collagenase (Sigma) for 30 min under constant agitation at 37 °C. The suspension was washed in 10% FCS–RPMI-1640 medium. The remaining tissue was mechanically disrupted as previously described and washed in 10% FCS–RPMI-1640 medium (250 ***g*** for 5 min). The cell suspension was then passed through a packed glass-wool column to remove tissue debris and clumps of cells. After a further wash in 10% FCS–RPMI-1640 medium (250 ***g*** for 5 min), the cell suspension was washed in 100% FCS for 3 min at 100 ***g*** followed by 10% FCS–RPMI-1640 medium as described above. The cell suspension was transferred onto a Petri dish and incubated at 37 °C, 5% CO_2 for 1 h to allow adherent cells to stick to the plastic. The non-adherent cells were harvested and washed in 10% FCS–RPMI-1640 medium. Contaminating erythrocytes were lysed by treating with Tris–NH_4Cl and the cell suspension was then washed in 10% FCS–RPMI-1640 medium. Then 6 ml of the cell suspension were layered onto 3 ml of NycoPrep™ in a 15 ml centrifuge tube and centrifuged at 600 ***g*** for 15 min. The mononuclear cells formed a layer at the interface between the NycoPrep™ and the medium, with the hepatocytes pelleted at the bottom of the centrifuge tube. The lymphomyeloid cells (mononuclear cells) were washed in 10% FCS–RPMI-1640 medium. The final pellet of lymphomyeloid cells was resuspended in 10% FCS–RPMI-1640 medium for determination of cell number and viability. Peripheral blood was harvested from mice by cardiac puncture into a syringe containing sodium heparin (1000 i.u./ml) in PBS. The whole blood was then diluted by the addition of an equal volume of 0·9% sodium chloride and then subjected to density centrifugation. Six ml of the diluted blood were layered over 3 ml of NycoPrep™ in a 15 ml centrifuge tube and centrifuged at 600 ***g*** for 15 min. The peripheral blood mononuclear (PBMN) cells were harvested from the interface between the plasma layer and the NycoPrep™ solution. The PBMN cells were washed twice in 10% FCS–RPMI-1640 medium and then resuspended in 10% FCS–RPMI-1640 medium for determination of cell number and viability.

Cytological analysis of cell suspensions

Cell suspensions were prepared as described, adjusted to a final volume of 10^5 cells/ml in 10% FCS–RPMI-1640 medium and 500 μl of the cell suspension were aliquoted into the sample chamber and placed in the cytofuge (Shandon). The samples were centrifuged at 4000 ***g*** for 10 min. The microscope slide was air-dried, fixed in methanol and stained with Giemsa. The cells were counted under oil immersion ($\times 1000$). At least 500 cells per slide were counted.

Irradiation of mice

Mice were irradiated with a sublethal dose of 400 rad. from a ^{60}Co source chamber (Nuclear Engineering) in the Department of Veterinary Physiology, University of Glasgow. Irradiation of recipient mice occurred no earlier than 24 h prior to adoptive transfer and challenge infection.

Statistical analysis

Results are expressed as means $\pm$ 1 S.D. and group data were compared using a Student's *t*-test. A result was considered to be significant when the value of $P < 0{\cdot}05$.

RESULTS

IFNγR deficient mice are more susceptible to P. chabaudi *infection than IL-4 deficient mice*

It was found that 75% of IFNγR deficient mice succumbed to infection with 1×10^5 pRBCs of *P. chabaudi* and 43% died following infection with 1×10^1 pRBCs of *P. chabaudi* (Fig. 1). No mortality was observed in IL-4 deficient mice or control mice with either dose of *P. chabaudi* infection.

Infection of IFNγR deficient mice with either/or 1×10^5 pRBCs (Fig. 2A) and 1×10^1 pRBCs (Fig. 3) of *P. chabaudi* resulted in a significant secondary peak of parasitaemia (no recrudescence parasitaemia was observed in the control mice) observed after 20 days post-infection (p.i.) in the mice which survived (Figs 2A and 3). There was no significant difference between the IFNγR deficient mice and control mice during the primary parasitaemia although, con-

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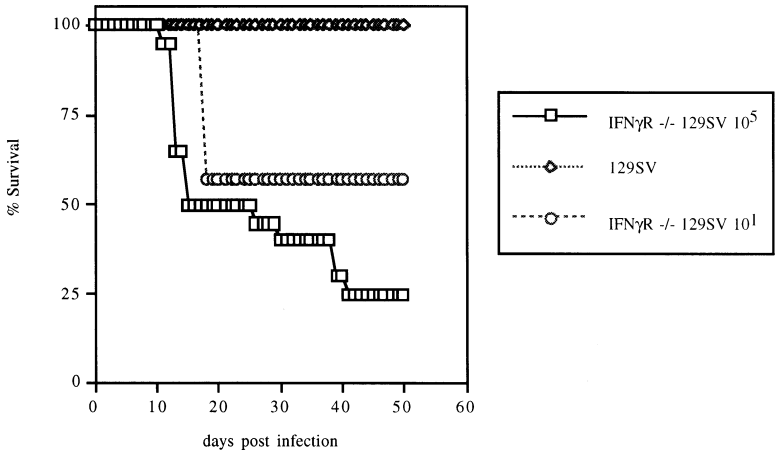

Fig. 1. *Plasmodium chabaudi* infection of IFNγR deficient mice results in an increased mortality rate. IFNγR deficient mice (IFNγR-/-129SV) and control mice (129SV) were infected with 1×10^5 or 1×10^1 pRBCs of *P. chabaudi*. Each group consisted of 6 mice and the results are combined from 3 replicate experiments.

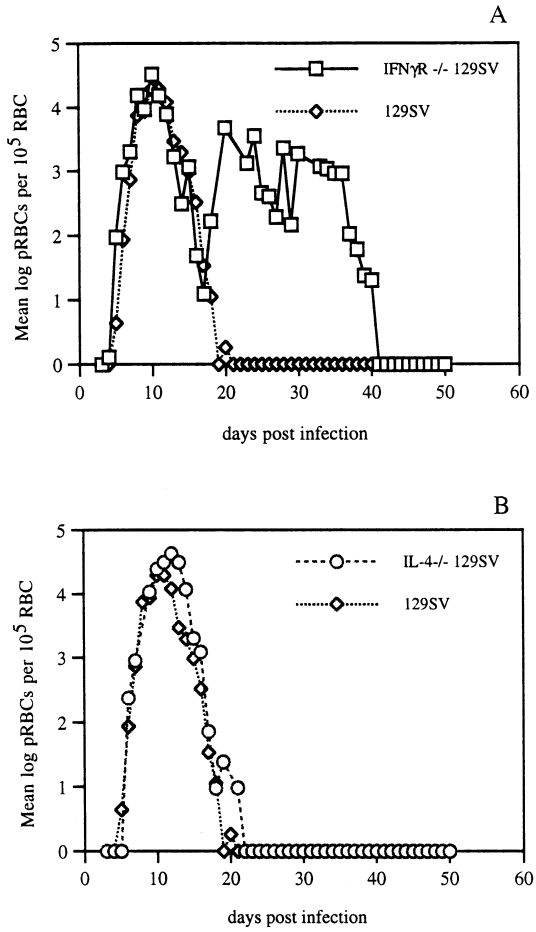

Fig. 2. IFNγR deficient mice are more susceptible to *Plasmodium chabaudi* infection than IL-4 deficient mice. IFNγR deficient mice, IL-4 deficient mice and control mice were infected with 1×10^5 pRBCs of *P. chabaudi*. The mean log parasitaemia for both IFNγR deficient mice (A) and IL-4 deficient mice (B) compared to control mice are presented. Each group consisted of 6 mice and the results are combined from 3 replicate experiments.

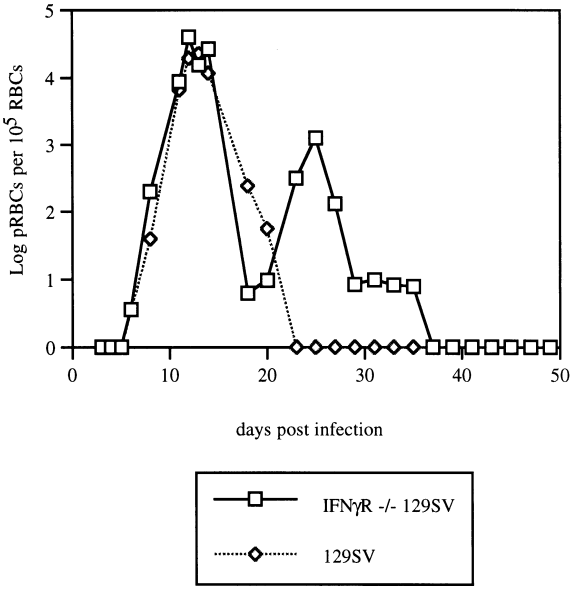

Fig. 3. IFNγR deficient mice are susceptible to a low infective dose of *Plasmodium chabaudi*. Both IFNγR deficient mice and control mice were infected with 1×10^1 pRBCs of *P. chabaudi*. The mean log parasitaemia of 6 mice per group are presented.

sistently, the peak of the primary parasitaemia was greater in the IFNγR deficient mice. In control mice the infection became subpatent around 20 days p.i. whereas in surviving IFNγR deficient mice, clearance of parasites to subpatent levels did not occur until approximately day 40 p.i.

IL-4 deficient mice controlled a *P. chabaudi* infection in a similar manner to control mice (Fig. 2B). A small but statistically significant exacerbation at peak parasitaemia (12 days p.i., $P < 0{\cdot}002$) was observed in the IL-4 deficient mice. This result was confirmed in further experiments using 2 different background strains, BALB/c and B6 × 129 (data not shown).

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Table 1. Parasite-specific IgG production in *Plasmodium chabaudi* AS infected INFγR deficient mice and IL-4 deficient mice

(Results are the mean of 3 samples and are expressed as the reciprocal of antibody titre (range of samples analysed was 1:50–1:1000).)

Days post-infection	Group IFNγR-/- 129SV	IL-4-/- 129SV	129SV
0	—	—	—
6	—	100	—
10	50	100	100
13	100	1000	1000
15	100	500	1000
22	N/A	1000	1000

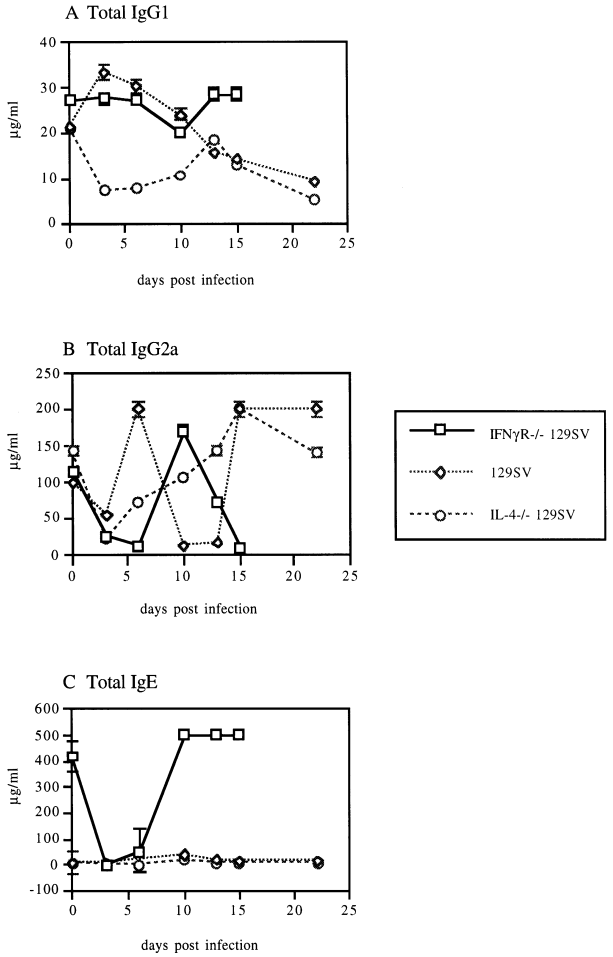

Fig. 4. Total antibody production detected in the serum of *Plasmodium chabaudi* infected IFNγR deficient mice and IL-4 deficient mice. Levels of total IgG1 (A), IgG2a (B) and IgE (C) were determined in the serum of IFNγR deficient mice, IL-4 deficient mice and control mice following inoculation with 1×10^5 pRBCs of *P. chabaudi*. Serum from 3 mice was analysed individually for each group, in triplicate and the mean ± S.D. was calculated.

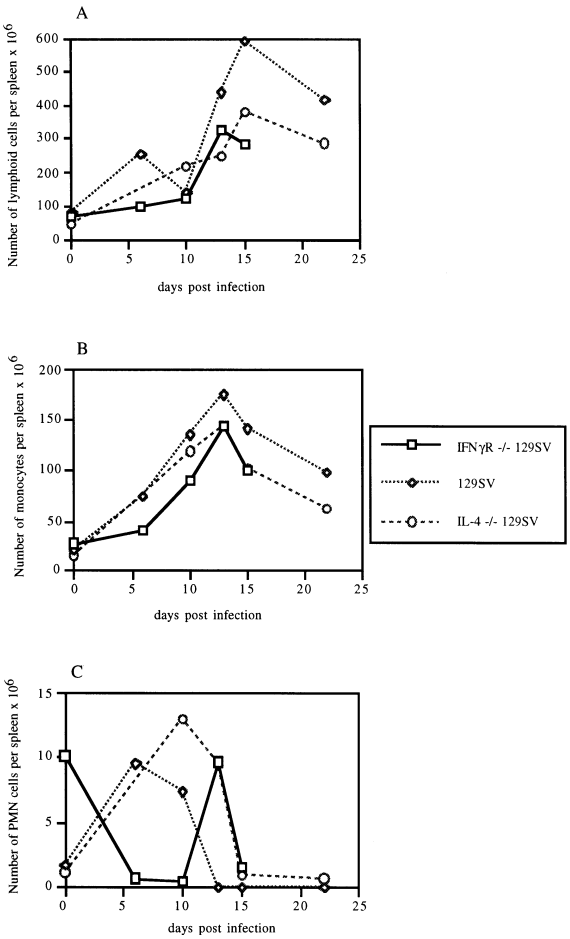

Fig. 5. Cytological analysis of cells present in the spleen of IFNγR deficient mice, IL-4 deficient mice and control mice following infection with 1×10^5 pRBCs of *Plasmodium chabaudi*. Three mice from each group were sacrificed at each time-point and the cells harvested and centrifuged. In total 500 cells were counted on each Giemsa-stained cytospin smear and the number of (A) lymphoid, (B) monocyte and (C) PMN cells expressed per spleen. The data shown are representative of 2 experiments.

Humoral response to P. chabaudi *AS infection in IFNγR deficient and IL-4 deficient mice*

The level of parasite-specific IgG in the serum of IFNγR deficient mice during *P. chabaudi* infection was reduced compared to control mice throughout the period of infection analysed (Table 1). Total IgG1 levels in IFNγR deficient mice were similar to that of control mice but there was a delay and a reduction in the total IgG2a response of IFNγR deficient mice to *P. chabaudi* infection compared to control mice (Fig. 4A and B). IFNγR deficient mice produced a significant amount of total IgE in response to *P. chabaudi* infection whereas no significant levels of total IgE were observed in the serum of control mice (Fig. 4C).

No significant differences were observed between IL-4 deficient mice and control mice in terms of

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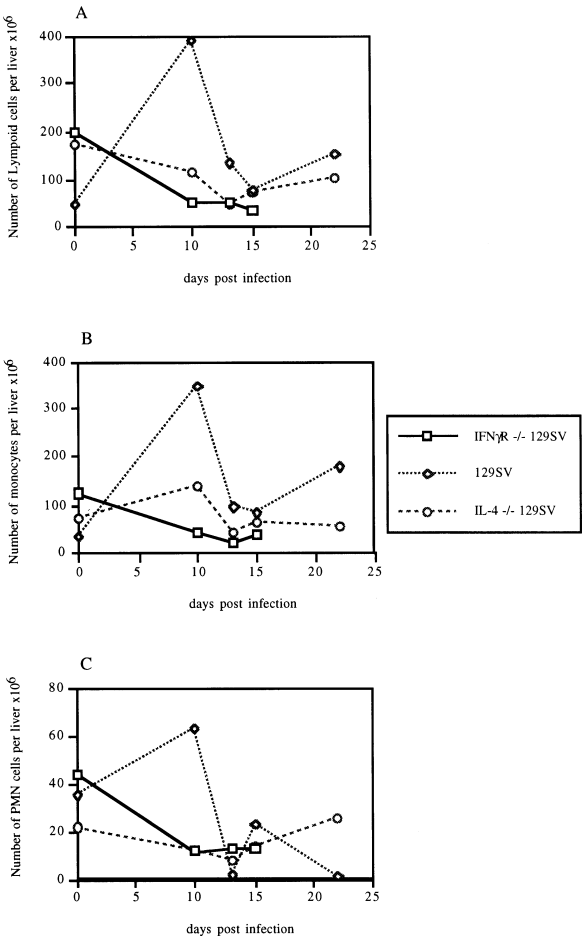

Fig. 6. Cytological analysis of cells present in the liver of IFNγR deficient mice, IL-4 deficient mice and control mice following infection with 1×10^5 pRBCs of *Plasmodium chabaudi*. Three mice from each group were sacrificed at each time-point and the cells harvested and centrifuged. In total 500 cells were counted on each Giemsa-stained cytospin smear and the number of (A) lymphoid, (B) monocyte and (C) PMN cells expressed per liver. The data shown are representative of 2 experiments.

parasite-specific IgG production (Table 1). Total IgG1 was reduced in IL-4 deficient mice compared to control mice but both groups had similar levels of total IgG2a (Fig. 4A and B). Neither IL-4 deficient mice nor control mice produced significant levels of total IgE (Fig. 4C).

Cytological analysis of cells present in the spleen and liver of P. chabaudi *infected mice*

A reduction in the number of lymphoid cells present in the spleen of IFNγR deficient mice when compared to control mice, was observed (Fig. 5). A similar trend was observed when comparing IL-4 deficient mice to control mice. No substantial differences were observed in the numbers of monocytes present in the spleen of both IFNγR deficient mice and IL-4 deficient mice during the period of

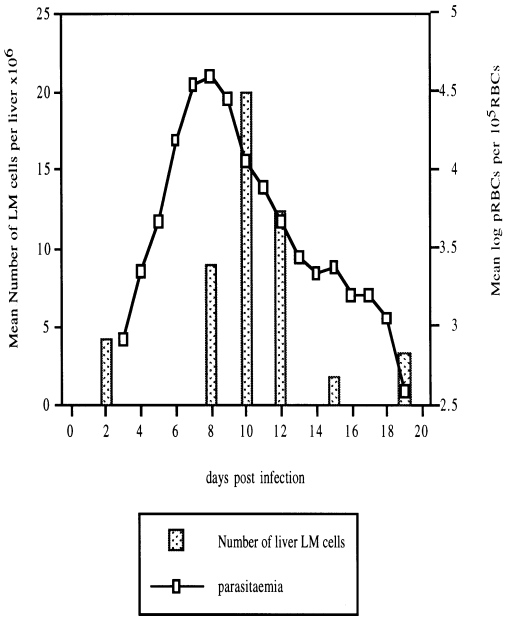

Fig. 7. Extraction of lymphomyeloid (LM) cells from the livers of *Plasmodium chabaudi* infected mice. Mice were infected with 1×10^6 pRBCs of *P. chabaudi* and 2 mice were sacrificed at each time-point. The mean number of cells per liver ($\times 10^6$) was calculated.

infection examined (22 days p.i.). IFNγR deficient mice had reduced numbers of PMN cells in the spleen compared to both IL-4 deficient mice and control mice during the period of days 6–10 p.i. Interestingly, there was a substantial reduction in the numbers of lymphoid cells, monocytes and PMN cells present in the liver of *P. chabaudi* infected IFNγR deficient mice and IL-4 deficient mice compared to infected control mice (Fig. 6). This coincided with exacerbation of infection in both IFNγR deficient mice (both parasitaemia and mortality rate) and IL-4 deficient mice (parasitaemia).

Isolation of lymphomyeloid (LM) cells from livers of P. chabaudi *infected mice and adoptive transfer studies*

To determine if liver LM cells participated in protective immune mechanisms to *P. chabaudi* in an immunocompetent host, we isolated liver LM cells from *P. chabaudi* infected NIH mice, a well-characterized model of *P. chabaudi* infection in our laboratory (Phillips, Mathers & Taylor-Robinson, 1994). Peak numbers of LM cells were isolated at day 10 p.i. (Fig. 7) similar to that observed in the control mice above (see Fig. 6).

Adoptive transfer studies were performed to determine if LM cells, isolated from the livers of infected mice could confer protection against a *P. chabaudi* challenge. Liver LM cells, non-adherent splenocytes and peripheral blood mononuclear cells (PBMN) were harvested from donor NIH mice at day 11 post-infection. Splenocytes were harvested

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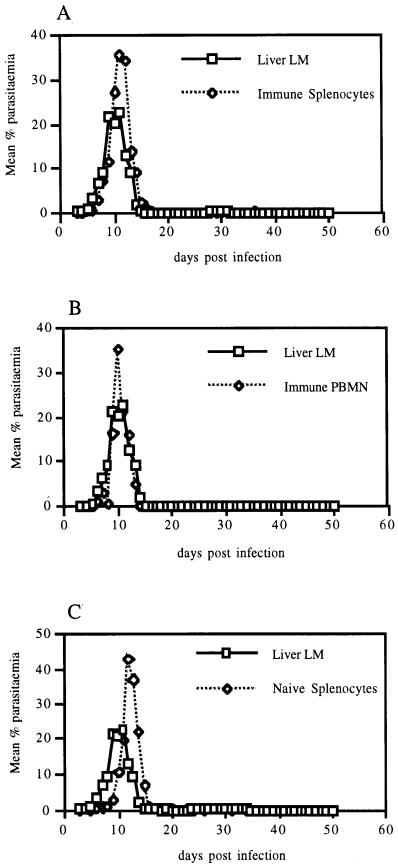

Fig. 8. Adoptive transfer of liver LM cells can confer protection to *Plasmodium chabaudi* infected recipient mice. Liver LM cells isolated at day 11 of a *P. chabaudi* infection and adoptively transferred to irradiated recipients, significantly reduced the peak of the primary parasitaemia of an homologous challenge compared with the transfer of (A) immune splenocytes [$P < 0{\cdot}04$], (B) immune peripheral blood monocytes (PBMN) [$P < 0{\cdot}04$] and (C) naïve splenocytes [$P < 0{\cdot}04$]. Each group of recipients consisted of 6 mice and were challenged with 1×10^5 pRBCs of *P. chabaudi*. The data shown are from 1 of 2 similar experiments.

from naïve NIH mice as a further control group rather than naïve liver LM cells due to the availability of cell numbers. Sublethally irradiated recipient NIH mice received either 3×10^6 liver LM cells, 3×10^6 PBMN cells, 1×10^7 immune splenocytes or 1×10^7 naïve splenocytes and all groups were subsequently infected i.v. with 1×10^5 pRBCs of *P. chabaudi*. Mice receiving the liver LM cells were significantly protected at peak parasitaemia when compared to the control groups (Fig. 8). No mortality was observed in any of the recipient groups.

DISCUSSION

The data presented in this study demonstrate that IFNγR deficient mice are more susceptible to *P. chabaudi* infection than mice deficient in IL-4 production. These observations confirm previous results (Favre *et al*. 1997; Van Der Heyde *et al*. 1997) illustrating the importance of IFNγ dependent cellular responses to malaria infection. A high mortality rate in the IFNγR deficient mice was observed whereas there were no deaths observed in control groups. Mortality was observed in IFNγR deficient mice when infected with as few as 1×10^1 pRBCs. The IFNγR deficient mice that survived the acute phase of infection had a pronounced secondary parasitaemia unlike the control mice where no recrudescence occurred during the period of infection observed.

It is unclear why there is a high mortality rate in IFNγR deficient mice following infection with the normally self-resolving *P. chabaudi*. Mortality is observed following infection with both 10^5 and 10^1 pRBCs (75 % and 43 % respectively) but appears not to be consistently linked with fulminating parasitaemia and occurs throughout the course of a primary infection. The extent of the anaemia in the IFNγR deficient mice was the same as control mice (Favre *et al*. 1997) and does not appear to contribute to the high mortality rate observed. It is unknown if there is an over-production of inflammatory mediators such as IL-1 or TNF to compensate for the lack of IFNγ stimulated inflammatory responses. This would be similar to *P. chabaudi* infection of IL-10 deficient mice which appear to succumb to an increase in pathology attributed to a combination of malaria toxins and an exacerbated inflammatory response (Linke *et al*. 1996).

A significant level of total IgE was observed in the serum of IFNγR deficient mice during *P. chabaudi* infection. IgE has recently been proposed as a pathogenic factor in *P. falciparum* infection (Perlmann *et al*. 1997). Elevated IgE levels have been observed in cerebral malaria patients compared to those with non-complicated malaria (Perlmann *et al*. 1994) and it is thought that the interaction of IgE with FcεRII (CD23) on various cells, including macrophages, eosinophils and B cells may induce the over-production of inflammatory cytokines which have been implicated in malarial pathology (Grau *et al*. 1987; Clark, Rockett & Cowden, 1991). There does not appear to be a correlation between the level of parasite-specific IgE and pathogenicity but rather, it is the quantity of IgE that correlates with disease severity (Perlmann *et al*. 1994, 1997). Hence, the significant total IgE response in *P. chabaudi* infected IFNγR deficient mice, may contribute to the pathology observed (high mortality rate) via the cross-linking of CD23, resulting in the over-production of pathogenic inflammatory cytokines.

IL-4 deficient mice, can control and clear a primary infection of *P. chabaudi* with similar efficiency to that of control mice. This observation confirms previous studies (Van Der Heyde *et al*. 1997; Von Der Weid *et al*. 1994) illustrating that the presence of IL-4 is not essential for the efficient

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elimination of a patent erythrocytic stage malarial infection in mice. However, a small but statistically significant exacerbation of the peak of the primary patent parasitaemia in the IL-4 deficient mice (on 3 different backgrounds) compared to control mice, was observed in this study which has not been previously reported.

The pronounced secondary peak of parasitaemia observed in the IFNγR deficient mice infected with *P. chabaudi* is in accord with a previous report (Favre *et al*. 1997) and is similar to the outcome of infection of IFNγ deficient mice with *P. chabaudi adami* in that clearance of infection is significantly delayed when compared to controls (Van Der Heyde *et al*. 1997). Mice treated with anti-IL-12 antibodies have also been shown to have a pronounced secondary parasitaemia following *P. chabaudi* infection (Yap, Jacobs & Stevenson, 1994) which when taken together with the results reported here and previous studies (Favre *et al*. 1997; Van Der Heyde *et al*. 1997) supports the hypothesis that IL-12 mediated protection is partly dependent upon IFNγ stimulated cellular responses (Stevenson *et al*. 1995).

The failure of IFNγR deficient mice to clear the infection as efficiently as control mice could be attributed to a deficiency in IFNγ dependent humoral responses. In this study, there is a reduction in parasite-specific IgG production and total IgG2a levels in the IFNγR deficient mice. IgG2a has been proposed to have a protective role in experimental malaria infection (Waki *et al*. 1995). It is possible that during the acute phase of infection, the parasite-specific isotype produced is IgG2a, an IFNγ dependent isotype, which could explain the lower level of parasite-specific antibody production in IFNγR deficient mice and the failure to resolve a *P. chabaudi* infection efficiently.

Both IFNγR deficient mice and IL-4 deficient mice have reduced numbers of liver LM cells during the acute phase of *P. chabaudi* AS infection. Subsequent experiments demonstrated that an increase in the numbers of liver LM cells occurs around the peak of the primary parasitaemia in an immunocompetent host and adoptive transfer of these cells could protect against an homologous infection. Previous studies have shown an increase in numbers of cells present in the liver during *P. chabaudi* infection (Kumararatne *et al*. 1987) and associated this increase with protection (Dockrell, De Souza & Playfair, 1980). Hence, the liver may be a site of a protective immune response to experimental blood-stage malaria infection.

Malaria is a very dynamic, systemic infection which induces the production of various inflammatory cytokines (Bate, Taverne & Playfair, 1988). Associated with the production of these cytokines, such as IL-1 and TNF, is the up-regulation of adhesion molecules (Schofield *et al*. 1996). The presence of the parasite induces this inflammatory environment but both the parasite and the immune system utilize it, either in the process of parasite sequestration or the recruitment of immune effector cells to various sites. In the *P. chabaudi* model, an important site of sequestration is the liver (Cox, Semof & Hommel, 1987). This process is linked to antigenic variation and the establishment of chronic infection (Gilks, Walliker & Newbold, 1990) but it is unclear if sequestration is required to induce the recruitment of LM cells to the liver. Infection of mice with a non-sequestering population of *P. chabaudi* would determine if sequestration is required for LM cell migration to the liver.

Both IFNγ and IL-4 can influence the expression of adhesion molecules which could reduce not only the level of sequestration of the parasite but reduce the recruitment of cells to the liver. This is reflected in the reduced numbers of liver LM cells observed in the liver of *P. chabaudi* infected IFNγR deficient mice and IL-4 deficient mice and may contribute to an increase in parasite survival. The IFNγR deficient mice do have an increased leukocytosis compared to control mice following a *P. chabaudi* infection (Favre *et al*. 1997 and personal observations) possibly as a consequence of a disruption in the redistribution of immune cells to organs such as the liver or spleen which has been previously reported during a *P. chabaudi* infection (Kumararatne *et al*. 1987).

The data reported in this study raise 2 interesting points. The first is that both IFNγR deficient mice and IL-4 deficient mice have reduced liver LM cell numbers yet only the IFNγR deficient mice succumb to infection. This suggests that the liver LM cells contribute only partially to parasite clearance at peak parasitaemia. Both IFNγR deficient mice and IL-4 deficient mice have exacerbated parasitaemia at this time, however, the absence of IFNγ mediated responses appears to be more detrimental to the development of protective immunity than the absence of IL-4. Secondly, Th2 responses are thought to control parasite elimination at the later stages of infection yet IL-4 deficient mice do not succumb to an exacerbation of infection at this time. This observation may be due to compensatory mechanisms in the IL-4 deficient mice which ensure that the absence of IL-4 does not result in the loss of the required immune response for elimination of the parasite. It has been previously suggested that IL-13 may replace the function of IL-4 *in vivo* (Von Der Weid *et al*. 1994).

The work reported in this study has confirmed the importance of IFNγ mediated immune responses during *P. chabaudi* infection and has demonstrated that LM cells, present in the liver during infection are capable of mediating anti-parasite effector mechanisms. The infection of IFNγR deficient mice with *P. chabaudi* provides a model which may contribute to the understanding of the development of protective immunity to blood-stage malaria.

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