International Immunology, Vol. 7, No. 6, pp. 1005–1011

αβ and γδ T cells in the immune response to the erythrocytic stages of malaria in mice

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Key words: malaria, *Plasmodium chabaudi*, T cell

Abstract

Mice lacking T cells with αβ TCR (TCR β–/–) or γδ TCR (TCR δ–/–) were infected with the erythrocytic stages of the malaria parasite, *Plasmodium chabaudi chabaudi* (AS). Mice without γδ T cells could control and reduce a primary infection of *P. chabaudi* with a slight delay in the time of clearance of the acute phase of infection and significantly higher recrudescent parasitaemias compared with control intact mice. TCR δ–/– mice had higher levels of both serum Ig and malaria-specific antibodies of the isotypes IgG3 and IgG1 compared with control mice. TCR β–/– mice, despite a striking increase in NK1.1$^+$ cells and the presence of γδ T cells, were unable to clear their infection. Although the plasma of TCR β–/– mice contained all Ig isotypes before and during a primary infection, they were unable to produce significant levels of malaria-specific IgG antibodies, suggesting that in the absence of αβ T cells γδ T cells are not able to provide efficient help for antibody production.

Introduction

T cells that express a γδ TCR constitute only a small minority of peripheral T cells in mouse and man (1–3), but have been often associated with a variety of infectious and parasitic diseases including malaria (4–8). An elevation in the numbers of γδ T cells has been observed in the peripheral blood and spleens of individuals with an acute or convalescent *Plasmodium falciparum* infection (7,9,10) and during fever paroxysm associated with a *P. vivax* infection (11). However, the reasons for their expansion and the role they play in immunity to malaria are not known.

The importance of CD4$^+$ T cells in the protective immune response to erythrocytic stage parasites in experimental models is well accepted (12–15). However, a contribution of other types of lymphocytes, particularly in the acute phase of a *P. chabaudi* infection, cannot be ruled out. In order to determine the possible functions of γδ T cells in malaria, mouse models of infection have been studied (5,16–18). An increase in the number and proportion of γδ T cells in the spleens of mice infected with *P. chabaudi chabaudi*, *P. chabaudi adami* and *P. yoelii* has been reported (16–18). This is particularly pronounced in mice which are deficient in β_2-microglobulin and hence lack most CD8$^+$ T cells (16) and in B cell-deficient mice (19). Furthermore, in mice lacking CD8$^+$ T cells or in adoptive transfer experiments, there is some evidence that γδ T cells can contribute to the reduction of parasitaemia (16,17). Human γδ T cells have been shown to inhibit the growth of *P. falciparum in vitro* (20).

The availability of mice lacking subsets of lymphocytes due to targeted gene disruption allows the direct evaluation of the roles of different cells in the immune response to rodent malarias. Using mice lacking αβ T cells (TCR β–/– mice, 21) or lacking γδ T cells (TCR δ–/– mice, 22) it has been demonstrated that γδ T cells can control to some extent a pre-erythrocytic infection of *P. yoelii* in mice immunized with irradiated sporozoites (23). Here, we have investigated whether there is any indication of a role for γδ T cells in the clearance of a primary infection with the erythrocytic stages of *P. chabaudi chabaudi* and in the production of malaria-specific antibodies. Our results indicate that γδ T cells alone are not able to control a primary erythrocytic infection of *P. chabaudi* malaria and are very inefficient as helper cells for B cell production of malaria-specific IgG antibodies *in vivo*.

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Transmitting editor: E. Simpson

Received 24 January, *accepted* 6 March 1995

Methods

Parasites and mice

Female mice homozygous for the targeted mutations of the TCR β and TCR δ genes (22,23) and their respective wild-type or heterozygous littermates (F_2(C57Bl/6×129) were used in these studies. They were bred and maintained in conventional facilities, using sterilized water, bedding and food, at the Max-Planck-Institut für Immunbiologie. Mice aged 6–10 weeks were infected with *P. chabaudi chabaudi* (AS) by i.p. inoculation of 10^5 infected erythrocytes as described previously (13,14). The course of infection was monitored by examination of Giemsa-stained blood smears performed three times a week throughout the experiment.

Antibodies to mouse lymphocyte surface markers

mAb specific for mouse TCRβ, TCRδ, CD3, CD4, CD8 and NK cells labelled with either biotin, fluorescein or phycoerythrin were obtained from PharMingen (San Diego, CA); polyvalent fluorescein-labelled goat anti-mouse IgG, streptavidin–fluorescein and streptavidin–phycoerythrin from Dianova (Hamburg, Germany); and streptavidin–Tricolor from Medac (Hamburg, Germany). mAb specific for B220 (RA33A.1) and labelled with biotin or fluorescein were prepared from cultures grown in the laboratory. For use antibodies were diluted in PBS containing 1% BSA, 0.1% NaN_3 and 0.05 mM EDTA (sorter buffer)

Flow cytometric analysis

Splenic cells and lymph node cells or purified T cell fractions thereof (see below) from infected or control mice were incubated sequentially with biotin-, fluorescein- or phycoerythrin-labelled antibodies in the presence of anti-FcR antibodies (PharMingen). Each incubation was for 30 min on ice, followed by two washes in sorter buffer. In the case of three-colour staining, streptavidin–Tricolor was added together with the last antibody. Two- and three-colour flow cytometry was carried out and analysed with a FACScan (Becton Dickinson, Mountain View, CA) using Becton Dickinson Lysys software. Viable lymphoid cells were gated using forward and 90° scatter.

ELISA assays

Circulating mouse IgM, IgG1, IgG2a, IgG2b, IgG3 and IgE were measured in the sera of malaria-infected mice using goat anti-mouse-IgM (Paesel, Frankfurt, Germany), -IgG1, -IgG2a, -IgG2b, -IgG3 and -IgE (Nordic Immunology, Bochum, Germany) respectively as coating antibody. The appropriate mouse Ig isotypes (IgM, IgG1, IgG2a, IgG2b, IgG3, Sigma, Deisenhofen, Germany; IgE, PharMingen) were used as standards. The specificity of the isotype reagents was tested by using the standards of all the other isotypes as negative controls up to a concentration of 500 μg/ml.

In addition, malaria-specific antibodies of the various isotypes, except IgE, were measured in the sera of mice by using a lysate of the erythrocytic stages of *P. chabaudi* as the coating antigen (24). Hyperimmune serum obtained from mice challenged several times with a high dose of *P. chabaudi*-infected erythrocytes was used as standard. Goat anti-mouse-IgM, -IgG1, -IgG2a, -IgG2b, -IgG3 (Southern Biotechnology, Hamburg, Germany) and -IgE (PharMingen) conjugated with alkaline phosphatase were used to detect specifically bound mouse Ig of the respective isotypes. For the malaria-specific isotypes, the results are expressed as $\log_{10}$ values of arbitrary units as calculated from a standard hyperimmune serum, where the hyperimmune serum was assigned an arbitrary value of 10,000 units for each isotype. It should be noted that the units of malaria antibody are relevant only for purposes of comparison within a particular isotype and should not be used to compare the relative levels of the different isotypes.

Results

Course of a *P. chabaudi* infection in TCR β−/− and TCR δ−/− mice

TCR β−/− mice and sex-matched heterozygous littermate controls were infected with 10^5 *P. chabaudi*-infected erythrocytes (Fig. 1). Although these mice are F_2(C57BL/6×129), the course of the initial infection in the heterozygous littermate controls resembles that observed previously in female C57BL/6 mice (13). The larger SEM parasitaemia values may reflect this mixed background. Peak parasitaemias of 30–45% were observed within 9 days after inoculation of parasites. This acute infection was resolved within 25 days. In contrast to BL/6 and BALB/c mice (13,14), no recrudescent parasitaemias >0.02% were recorded within the 45 day observation period except in a single mouse (not shown in Fig. 1). The TCR β−/− mice were unable to clear their parasites <5% and generally the parasitaemias remained between 10 and

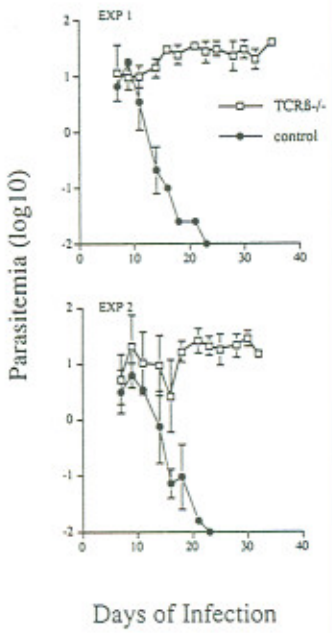

Fig. 1. Courses of a primary infection of *P. chabaudi* in female TCR β−/− and heterozygous control mice. Two independent experiments are shown. In the upper graph, the error bars represent the SEM of four control mice and five TCR β−/− mice. In the lower graph the SEM are calculated from the geometric mean parasitaemias of five TCR β−/− and five control mice.

40% for the duration of the experiment. Only one of 11 mice in the two experiments shown died (day 46) during the observation period of 60 days. Therefore in the absence of αβ T cells mice are unable to clear their parasites but do not die of fulminating parasitaemias.

Female TCR δ−/− and control mice were infected as described above. These mice are also of a mixed genetic background. Two experiments are shown (Fig. 2). In all cases TCR δ−/− mice were able to clear their parasitaemias to subpatent levels (<0.001% parasitaemia) within 40–50 days after the first infection. The peak of parasitaemia (15–35%) at 8–9 days after injection of 10^5 parasites was not significantly higher than that of the heterozygous control mice. However, these parasitaemias were maintained 3–4 days longer and were reduced to <0.001% at 3–7 days later than the controls. In the experiments shown, the parasitaemias were monitored for 60 days; three of nine and three of six TCR δ−/− mice in experiments 1 and 2 respectively exhibited substantial patent recrudescences of up to 6% which were later cleared. In contrast, only one heterozygous mouse (experiment 2) had a patent recrudescence. These data show that mice lacking γδ T cells can clear their infections as do their littermate controls. However, they also suggest that γδ T cells may contribute to reducing the period of acute infection and to controlling recrudescences.

Flow cytometric analysis

Splenic cells from TCR β−/−, TCR δ−/− and control mice were analysed by flow cytometry throughout a primary infection of *P. chabaudi*. Spleens of C57Bl/6 and other strains of mice increase in size during a primary erythrocytic infection with *P. chabaudi* (18) as with other mouse malarias (25), and this was observed also in all three groups of infected mice in these experiments. The nucleated cell content of the spleens of TCR β−/− mice was somewhat variable before infection (Table 1) but their numbers increased substantially during infection (9-fold after 60 days of chronic parasitaemia). A smaller increase in total numbers of cells was observed in the control mice (Table 1) and the TCR δ−/− mice (not shown), which returned to normal levels after reduction of the parasitaemia (3–4 weeks).

Apart from the absence of γδ T cells in the TCR δ−/− mice, there were no differences between TCR δ−/− and control mice in the major lymphoid populations of the spleen (data not shown). Flow cytometric data from TCR β−/− mice and controls are shown Table 1. During the acute infection in intact animals (between days 10 and 18) and throughout infection in the TCR β−/− mice, ~40% of the viable nucleated cells could not be identified by any of the antibodies used. This is in agreement with previously published findings (18,25) and is thought to reflect the increased haematopoeisis occurring after erythrocyte destruction by the parasite. Since the parasitaemia is not cleared in TCR β−/− mice, there is prolonged splenomegaly due to the production of these cells. This results in a reduction in the relative proportion of B cells at this stage of infection (5.6% compared with 43% in control mice which have cleared their infection).

Although γδ T cells increase in total numbers and proportion in TCR β−/− mice after prolonged infection (3% in uninfected mice increasing to 8.6% after 60 days), $CD3^+$ T cells remained at <10% of the analysed cell population. Interestingly, after 60 days of chronically high parasitaemias in the TCR β−/− mice, the major cell population stained positively with the NK1.1 antibody (up to 35% of the total gated cell population) suggesting an increase in the numbers of NK cells in the spleens of infected TCR β−/− mice.

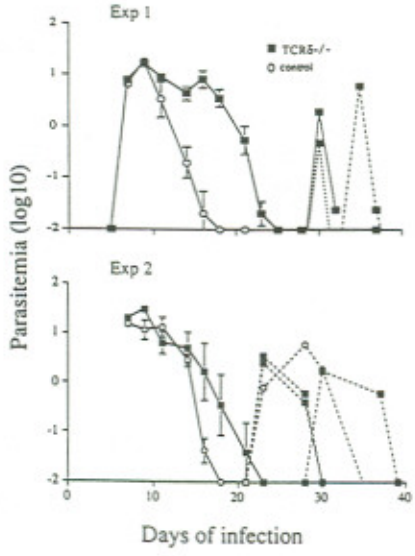

Fig. 2. Courses of a primary erythrocytic infection of *P. chabaudi* in female TCR δ−/− and control mice. SEM in the upper graph were calculated from four heterozygous and nine TCR δ−/− mice, and in the lower graph from five heterozygous and six TCR δ−/− mice. The infections indicated by the dotted lines are of individual TCR δ−/− and control mice.

Total plasma Ig levels in infected TCR β−/− and TCR δ−/− mice during a P. chabaudi infection

The circulating levels of Ig of the different isotypes were measured in the plasma of uninfected TCR β−/− and TCR δ−/− mice, and at intervals during a primary infection. A representative experiment is shown in Fig. 3. IgM was present in the plasma in similar amounts in uninfected TCR β−/− TCR δ−/− and control mice. Whereas the amounts of IgM increased somewhat in controls and TCR δ−/− mice during the primary infection, IgM in the TCR β−/− mice remained at the same level or decreased. All the IgG isotypes were present at low amounts in uninfected TCR β−/− mice and did not increase during infection. IgE could not be detected at any time in these mice.

Plasma of uninfected TCR δ−/− mice contained IgG of all isotypes. IgG1, IgG2a and IgG3 were at significantly higher concentrations compared with control mice. At 14 days of infection there were increases in the all IgG isotypes compared with uninfected mice. The levels dropped by day 28. Similar but less marked increases of IgG3 and IgG2a were observed in control mice. The increase in IgG1 was comparable in both controls and TCR δ−/− mice. IgE was present at detectable levels in uninfected TCR δ−/− (variable) but not in control mice. At 14 days of infection IgE became detectable also in control mice.

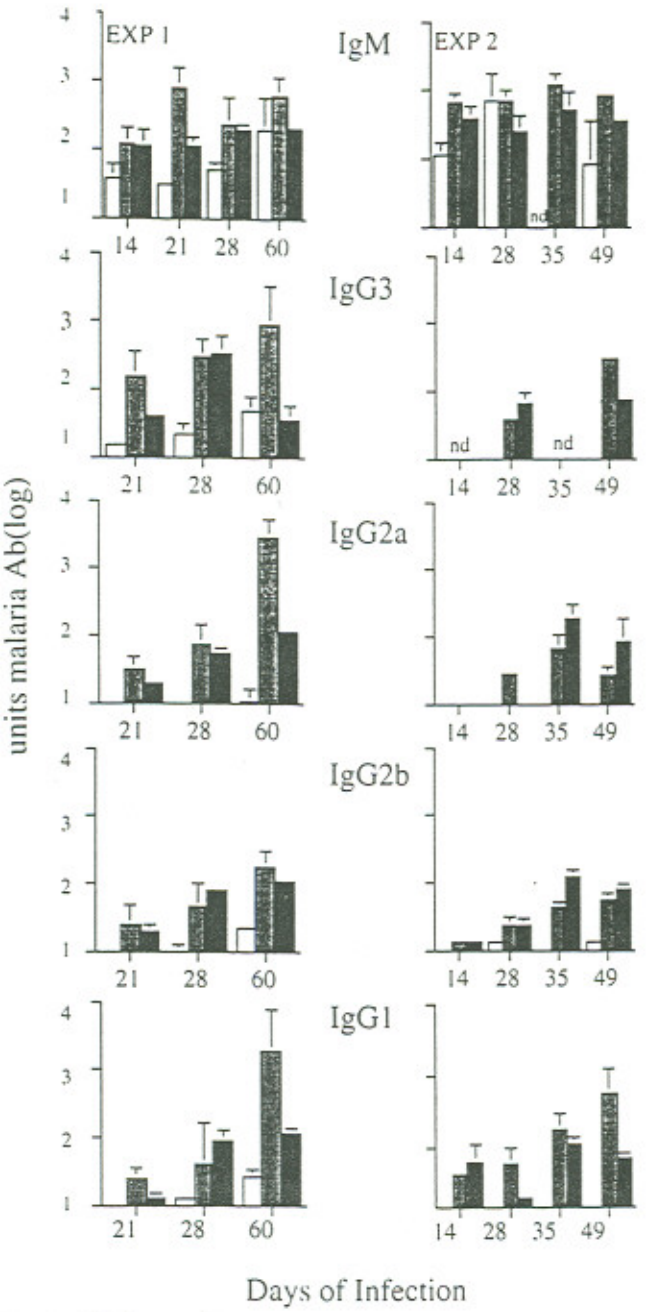

Fig. 4. Malaria-specific antibody responses of TCR δ−/− (open columns), TCR β−/− (shaded columns) and control mice (filled columns) at different times during a primary infection with *P. chabaudi*. The units of malaria-specific antibody were calculated as described in Methods and should be read only as comparisons of the amount of one isotype produced in the different mice. SEM shown are calculated from the geometric means of five to six individual animals at each time point as described in Fig. 3.

sensitivity responses and granuloma formation dependent upon IFN-γ production was found to be lacking in TCR β−/− mice infected by *Listeria monocytogenes* despite the ability of these mice to control their infections (35). In this case it was suggested that γδ T cells mediated their anti-listerial effects by means other than IFN-γ production.

γδ T cells can also be cytolytic (34,36), so it is possible that this is a mechanism for controlling the erythrocytic parasite stages. In this regard, it has been shown that human γδ T cells can inhibit the growth of *P. falciparum in vitro* with activity directed primarily against the extracellular merozoite (20). Clearly it will be of interest to determine whether mouse γδ T cells kill blood stage parasites similarly or whether their effects are mediated through activated macrophages. Our present data with *P. chabaudi* contrast to some extent with the finding that the course of *P. yoelii* blood stage infections are unaffected by the absence of γδ T cells (23). The small differences seen in the clearance of the acute *P. chabaudi* parasitaemia in mice with and without γδ T cells may reflect a more important role for antibody-independent immune mechanisms in this infection compared with *P. yoelii* infections as suggested previously (37).

The TCR β−/− mice did not die of a fulminating infection and, indeed, rarely died during the course of the experiments, suggesting that parasitaemias could be partially controlled in the absence of αβ T cells. The factors responsible for this control are not clear. The expansion of γδ T cells in *P. chabaudi*-infected mice *in vivo* has been shown to be dependent on $CD4^+$ T cells (17) and $CD4^+$ T cells or IL-2 are required for optimum growth of human γδ T cells *in vitro* (38). Therefore possible sub-optimal activation and thus lower numbers of effector γδ T cells may result in only partial control of parasitaemia. However, TCR β−/− mice have been shown to control a pre-erythrocytic infection of malaria (23) and a primary *Listeria* infection (35), suggesting that γδ T cells can be activated appropriately *in vivo* without αβ T cells.

The majority of identifiable cells in the spleens of chronically infected TCR β−/− mice were $NK1.1^+$ NK cells. These cells have been shown to produce IFN-γ, TNF-α and granulocyte macrophage colony stimulating factor (39,40), and are capable of activating macrophages to become tumoricidal (41) and able to kill intracellular pathogens such as *L. monocytogenes* (42). Thus it is likely that this T-cell-independent pathway of macrophage activation can control the parasitaemia to a limited extent. NK cells often appear early at sites of infection with *Listeria*, *Corynybacterium parvum* (43) and a variety of virus infections (44,45), and have been shown to expand in *nu/nu* and SCID mice infected with different pathogens (40). It has been proposed that the NK cell is a component of an innate mechanism of immunity against infection or altered self (39,40). Although we have not yet investigated the cytokine profiles of TCR β−/− mice infected with *P. chabaudi*, it is clear that this expanded NK population together with the γδ T cell in these mice are insufficient to resolve a primary parasitaemia.

Previous experiments describing a role for γδ T cells in the control of liver stage infections of *P. yoelii* (23) emphasize the essential differences in the immune responses effective against the different stages of *Plasmodium*. The elimination of liver stage parasites in certain strains of mice immunized with irradiated sporozoites is thought to be largely dependent on $CD8^+$ T cells, resulting in a cytotoxic response and/or production of IFN-γ (46,47). Presumably, γδ T cells could contribute significantly to the killing of intrahepatic parasites by similar mechanisms. *P. yoelii* erythrocytic stage parasites,

similar to our present findings with *P. chabaudi*, are not controlled in the absence of αβ T cells (23). Killing or destruction of erythrocytic stage parasites therefore requires additional factors that can only be provided by αβ T cells.

The erythrocytic infection of *P. chabaudi* in TCR β−/− mice allowed us to investigate whether γδ T cells are able to help B cells in the production of malaria-specific IgG antibodies. There is little direct evidence to date indicating that γδ T cells have this capacity, although it has been reported that γδ T cells may help in the production of autoantibodies (48), and the cytokines produced by some mouse and human γδ T cells such as IL-5 and IL-10 (2,49,50) are amongst those required for B cell differentiation and Ig production.

Our studies demonstrate that most Ig isotypes are present in the plasma of TCR β−/− mice. With the exception of IgM, they are present at much lower concentrations than in control mice. However, some switch to IgG is clearly possible in the absence of αβ T cells. These data contrast with the findings in TCR α−/− mice, where concentrations of all isotypes including IgE were comparable to the heterozygote controls (51). In those studies cytokines such as IL-4, able to promote the IgG1 and IgE switch, were detected. It is generally thought that the switch to IgG also requires a cognate interaction of gp39 on the T cell and CD40 on the B cell (52,53). gp39 was not found on the γδ T cells of the TCR α−/− mice (50). Therefore it was proposed that non-cognate interactions and the appropriate cytokines may have been responsible for the production of IgG in TCR α−/− mice. A possible explanation for the differences in IgG concentrations between the TCR β−/− and TCR α−/− mice could be that in the TCR α−/− mice there may be small numbers of T cells with TCR composed of β chain homodimers that are able to provide the necessary signals and/or cytokines. Our data in the TCR β−/− mice so far would support some non-antigen driven oligoclonal expansion and maturation of B cells, since despite the presence of circulating IgG we were unable to measure significant levels of T cell-dependent malaria-specific IgG antibodies. This is similar to the situation in 'leaky' SCID mice which have sometimes high levels of Ig in the plasma but do not make specific 'T cell-dependent' antibody responses (54).

Ig of all isotypes was present in the plasma of TCR δ−/− mice at levels similar to or higher than intact control mice. This was reflected to some extent in the specific antibody response, where IgG3, IgG1 and, to a lesser extent, IgG2a, were present in higher amounts. A regulatory role for γδ T cells on Ig production or particular Ig isotypes has not been described, but it is feasible that they may influence the microenvironment through the cytokines they produce such that particular isotypes are favoured, or that development of T_h1 or T_h2 $CD4^+$ T cells is differentially regulated. We are currently investigating the cytokine responses in these *P. chabaudi*-infected TCR β−/− and TCR δ−/− mice.

In summary, these studies indicate a minor role for γδ T cells and show the importance of αβ T cells in clearing an acute infection of *P. chabaudi*. It appears that γδ T cells are not effective alone in providing help for the generation of malaria-specific antibodies, but they may influence the quality and quantity of Ig produced. These mice will be useful tools with which to investigate the roles of αβ and γδ T cells in the development of malarial pathology.

Acknowledgements

We would like to thank Sabine Schindler for excellent t nical assistance, and Lucia Casabò, Thierry von der Weid and phen Morris-Jones for critical reading of the manuscript. This w was supported by UNDP/World Bank/WHO programme for Rese and Training in Tropical diseases (TDR).

References

1 Falini, B., Flenghi, L., Pileri, S., Pelicci, P., Fagioli, M., nelli, M. F., Moretta, L. and Ciccone, E. 1989. Distribution o cells bearing different forms of the T cell receptor γ/δ in nor and pathological human tissues. *J. Immunol.* 143:2480

2 Haas, W., Pereira, P. and Tonegawa, S. 1993. Gamma/de cells. *Annu. Rev. Immunol.* 11:637.

3 Allison, J. P. 1993. γδ cell development. *Curr. Opin. Immun* 5:241

4 Modlin, R. L., Pirmez, C., Hofman, F. M., Torigian, V., Uyen a, K., Rea, T. H., Bloom, B. K. and Brenner, M. B. 1989. Lymp ytes bearing antigen-specific γδ T-cell receptors accumulate i man infectious disease lesions. *Nature* 339:544.

5 Minoprio, P., Itohara, S., Heusser, C., Tonegawa, S. and Coutinho, A. 1989. Immunology of murine *T. cruzi* i ion: the predominance of parasite non-specific responses d the activation of TCR1 T cells. *Immunol. Rev.* 112:183.

6 Hiramatsu, K., Yoshikai, Y., Matsuzaki, G., Ohga, S., Mura r, K., Matsumoto, K., Bluestone, J. A. and Nomoto, K. 1992. A p ctive role of γ/δ T cells in primary infection with *Listeria* monocy enes in mice. *J. Exp. Med.* 175:49.

7 Ho, M., Webster, H. K., Tongtawa, P., Pattanapanyasat and Weidanz, W. P. 1990. Increased γδ T cells in acute *Plas dium falciparum* malaria. *Immunol. Lett.* 25:139.

8 Russo, D. M., Armitage, R. J., Barral-Netto, M., Ba A., Grabstein, K. H. and Reed, S. G. 1993. Antigen-reactive γ cells in human Leishmaniasis. *J. Immunol.* 151:3712.

9 Roussilhon, C., Agrapart, M., Ballet, J.-J. and Bensu n, A. 1990. T lymphocytes bearing the γδ T cell receptor in ients with acute *Plasmodium falciparum* malaria. *J. Infect. Dis.* 283.

10 Bordessoule, D., Gaulard, P. and Mason, D. Y. 1990. Pre ential localization of human lymphocytes bearing γδ T cells r tors to the red pulp of the spleen. *J. Clin Pathol.* 43:461.

11 Pereira, M. K., Carter, R., Goonewardene, R. and Menc K. N. 1994. Transient increase in circulating γ/δ T cells uring *Plasmodium vivax* malarial paroxysms. *J. Exp. Med.* 179 1

12 Brake, D. A., Weidanz, W. P. and Long, C. A. 1986. gen-specific, interleukin-2 propagated T lymphcytes confer re ance to a murine malaria parasite, *Plasmodium chabaudi amii. J. Immunol.* 137:347.

13 Süss, G., Eichmann, K., Kury, E., Linke, A. and Langh e, J. 1988. Roles of $CD4^-$ and $CD8^-$ bearing T lymphocyte n the immune response to the erythrocytic stages of *Plas dium chabaudi. Infect. Immun.* 56:3081.

14 Meding, S. J. and Langhorne, J. 1991. $CD4^+$ T cells an cells are necessary in the transfer of protective immunity to *Plas dium chabaudi chabaudi. Eur. J. Immunol.* 21:1430

15 Podoba, J. E. and Stevenson, M. M. 1991. $CD4^+$ ar $CD8^+$ T lymphocytes both contribute to acquired immunity t lood-stage *Plasmodium chabaudi* (AS). *Infect. Immun.* 59:51

16 van der Heyde, H. C., Elloso, M. M., Roopenian, C., Manning, D. D. and Weidanz, W. P. 1993. Expansion of $CD4^-$ $CD8^-$ γδ T cell subset in the spleens of mice during blo stage malaria. *Eur. J. Immunol.* 23:1846.

17 van der Heyde, H. C., Manning, D. D. and Weidanz, W. 1993. Role of $CD4^+$ T cells in the expansion of the $CD4^-$ $CD8^-$ γδ T cell subset in the spleens of mice during blood-stage laria. *J. Immunol.* 151:6311.

18 Langhorne, J., Pells, S. and Eichmann, K. 1993. Ph otypic characterisation of splenic T cells from mice infec with *Plasmodium chabaudi chabaudi. Scand. J. Immunol.* 3 21.

19 von der Weid, T. and Langhorne, J. 1994. A dual role fo cells in *Plasmodium chabaudi chabaudi* (AS) infection? *Res. unol. (59th Forum in Immunology)* 145:412

20 Elloso, M. M., van der Heyde, H. C., van der We A.,

Manning, D. D. and Weidanz, W. P. 1994. Inhibition of *Plasmodium falciparum in vitro* by human γδ T cells. 153:1187.
21 Mombaerts, P., Clarke, A. R., Rudnicki, M. A., Iacomini, J., Itohara, S., Lafaille, J. J., Wang, L., Ichikawa, Y., Jaenisch, R., Hooper, M. L. and Tonegawa, S. 1992. Mutations in T-cell antigen receptor genes α and β block thymocyte development at different stages. *Nature* 360:225
22 Itohara, S., Mombaerts, P., Lafaille, J., Iacomini, J., Nelson, A., Clarke, A. R., Hooper, M.-L., Farr, A. and Tonegawa, S. 1993. T cell receptor γδ gene mutant mice: independent generation of αβ T cells and programmed rearrangement of γδ TCR genes. *Cell* 72:337.
23 Tsuji, M., Mombaerts, P., Lefrancois, L., Nussenzweig, R. S., Zavala, F. and Tonegawa, S. 1994. γδ T cells contribute to immunity against the liver stages of malaria in αβ T-cell-deficient mice. *Proc. Natl Acad. Sci. USA* 91:345.
24 Langhorne, J., Evans, C. B., Asofsky, R. and Taylor, D. W. 1984. Immunoglobulin isotype distribution of malaria-specific antibodies produced during infection with *Plasmodium chabaudi adamii* and *Plasmodium yoelii*. *Cell Immunol.* 87:452.
25 Freeman, R. R. and Parish, C. R. 1978 Polyclonal B cell activation during rodent malarial infection. *Clin. Exp. Immunol.* 32:41.
26 Grun, J. L. and Weidanz, W. P. 1981. Immunity to *Plasmodium chabaudi adami* in the B-cell-deficient mouse. *Nature* 290:143
27 Cavacini, L. A., Parke, L. A. and Weidanz, W. P. 1990. Resolution of acute malarial infections by T cell-dependent non-antibody dependent mediated mechanisms of immunity. *Infect. Immun.* 58:2946.
28 von der Weid, T. and Langhorne, J. 1993. Altered response of $CD4^+$ T cell subsets to *Plasmodium chabaudi chabaudi* in B-cell-deficient mice. *Int. Immunol.* 5:1343.
29 Rockett, K. A., Awburn, M. M., Cowden, W. B. and Clark, I. A. 1991. Killing of *Plasmodium falciparum in vitro* by nitric oxide derivatives. *Infect. Immun.* 59:3280.
30 Dockrell, H. M. and Playfair, J. H. L. 1983. Killing of blood-stage murine malaria parasites by hydrogen peroxide. *Infect. Immun.* 39:456.
31 Rockett, K. A., Awburn, M. M., Aggarwal, B. B., Cowden, W. B. and Clark, I. A. 1992. *In vivo* induction of nitrite and nitrate by tumor necrosis factor, lymphotoxin and interleukin-1: possible roles in malaria. *Infect. Immun.* 39:456.
32 Cavacini, L. A., Guidotti, M., Parke, L. A., Melancon-Kaplan, J. and Weidanz, W. P. 1989. Reassessment of the role of splenic leukocyte oxidative activity and macrophage activation in expression in immunity to malaria. *Infect. Immun.* 57:3677.
33 Porcelli, S. B., Brenner, M. B. and Band, H. 1991. Biology of the human γδ T cell receptor. *Immunol. Rev.* 120:137.
34 Spits, H., Paliard, X., Engelhard, V. H. and De Vries, J. W. 1990. Cytotoxic activity and lymphokine production of T cell receptor (TCR)-$\alpha\beta^+$ and TCR $\gamma\delta^+$ cytotoxic T lymphocyte (CTL) clones recognising HLA-A2 and HLA-A2 mutants. *J. Immunol.* 144:156.
35 Mombaerts, P., Arnoldi, J., Russ, F., Tonegawa, S. and Kaufmann, S. H. E. 1993. Different roles of αβ and γδ T cells in immunity against an intracellular pathogen. *Nature* 365:53.
36 Koizumi, H., Lin, C.-C., Zheng, L. M., Joag, S. V., Bayne, N. K., Holoshitz, J. and Young, J. D.-E. 1991. Expression of perforin and serine esterases by human γ/δ T cells. *J. Exp. Med.* 173:499.
37 Langhorne, J. 1994. The Immune response to the blood stages of Plasmodium in animal models. *Immunol. Lett.* 41:99.
38 Kjeldsen-Kragh, J., Quayle, A. J., Skålhegg, B. S., Sioud, M. and Førro, Ø. 1993. Selective activation of resting human γδ T lymphocytes by interleukin-2. *Eur. J. Immunol.* 23:2092.
39 Perussia, B. 1991. Lymphokine activated killer cells, natural killer cells and cytokines. *Curr. Opin. Immunol.* 3:49.
40 Bancroft, G. J., Sheehan, K. C. F., Schreiber, R. D. and Unanue, E. R. 1989. Tumor necrosis factor is involved in the T cell-dependent pathway of macrophage activation in SCID mice. *J. Immunol.* 143:127.
41 Trinchieri, G. 1989. Biology of natural killer cells. *Annu. Rev. Immunol.* 47:187.
42 Bancroft, G. J., Schreiber, R. D., Bosma, G. C., Bosma, M. J. and Unanue, E. R. 1987. A T-cell independent mechanism of macrophage activation by Interferon-γ. *J. Immunol.* 1104.
43 Wherry, J. C., Schreiber, R. D. and Unanue, E. R. 1991. Regulation of γ-Interferon production by natural killer cells in SCID mice: roles of tumour necrosis factor and bacterial stimulii. *Infect. Immun.* 59:1709.
44 Shandley, J. D. 1990. *In vivo* administration of monoclonal antibody to NK1. 1 antigen of natural killer cells: effects on acute murine cytomegalovirus infection. *J. Med. Virol.* 30:58.
45 Welsh, R. M., Brubaker, J. O., Vargascortes, M. and O'Donell, C. L. 1991. Natural killer (NK) cells response to virus infection in mice with severe combined immunodeficiency. The stimulation of natural killer cells and the NK cell-dependent control of virus infection occur independently of T and B cell function. *J. Exp. Med.* 173:1053
46 Scofield, L., Vilaquiran, J., Ferreira, A., Schellekens, H., Nussenzweig, R. and Nussenzweig, V. 1987. γ-Interferon, $CD8^+$ T cells and antibodies required for immunity to malaria parasites. *Nature* 330:664
47 Hoffman, S. L. and Franke, E. D. 1994. Inducing protective immune responses against the sporozoite and liver stages of malaria. *Immunol. Lett.* 41:89.
48 Rajagopalan, S., Zordan, T., Tsokos, G. C. and Datta, S. K. 1990. Pathogenic anti-DNA auto-antibody-inducing T-helper cell lines from patients with active hyponephritis: isolation of $CD4^-8^-$ T helper cell lines that express the γδ T cell antigen receptor. *Proc. Natl Acad. Sci. USA* 87:7020.
49 Eichelberger, M., Allan, W., Carding S. R., Bottomly, K. and Doherty, P. C. 1991. Activation status of the $CD4^-$ 8^- $\gamma\delta^-$ T cells recovered from mice with influenza pneumonia. *J. Immunol.* 147:2069.
50 Taguchi, T. Aicher, W. K., Fujihashi, K., Yamamoto, M., McGee, J. R. and Bluestone, J. A., 1991. Intestinal intra-epithelium lymphocytes murine $CD4^+$ γδ TCR^+ T cells produce IFN-γ and IL-5. *J. Immunol.* 147:3736.
51 Wen, L., Roberts, S. J., Viney, J. L., Wong, F. S., Mallick, C., Fingly, R. C. Peng, Q., Craft, J. E., Owen, M. J. and Hayday, A. C. 1994. Immunoglobulin synthesis and generalised autoimmunity in mice congenitally deficient in $\alpha\beta^{(+)}$ T cells. *Nature* 369:654.
52 Nonoyama, S., Hollenbaugh, D., Aruffo, A., Ledbetter, J. A. and Ochs, H. D. 1993. B cell activation via CD40 is required for specific antibody production by antigen-stimulated human B cells. *J. Exp. Med.* 179:1097.
53 Spriggs, M. K., Armitage, R. J. Stockbine, L., Clifford, K. N., MacDuff, B. M., Stao, T. A., Maliszewski, C. R. and Fanslow, W. C. 1992. Recombinant human CD40 ligand stimulates B cell proliferation and immunoglobulin E secretion. *J. Exp. Med.* 176:1543.
54 Gibson, D. M., Bosma, G. C. and Bosma, M. J. 1989. Limited clonal diversity of serum immunoglobulin in leaky scid mice. In Bosma, M. J., Phillps, R. A. and Schuler, W., eds, *The SCID Mouse. Characterisation and Potential Uses*, p. 125. Springer, Heidelberg.