Immunology 1992 77 99–105

Functional characterization of protective CD4$^+$ T-cell clones reactive to the murine malaria parasite *Plasmodium chabaudi*

A. W. TAYLOR-ROBINSON & R. S. PHILLIPS *Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Glasgow*

Accepted for publication 13 April 1992

SUMMARY

Protective immunity to asexual malaria parasites appears to be mediated predominantly by the CD4$^+$ subset of T lymphocytes. To examine the role of this T-cell population in the immune response to the murine malaria parasite *Plasmodium chabaudi*, CD4$^+$ clones derived from infected mice were raised and propagated *in vitro*. Analysis of the reactivity of clones responsive to parasite antigen demonstrated that the CD4$^+$ cell response is heterogeneous and is consistent with the idea of two functionally distinct CD4$^+$ subsets. Those populations derived early during primary infection secreted interleukin-2 (IL-2) and interferon-gamma (IFN-γ) upon antigenic stimulation *in vitro*, i.e. they had a cytokine repertoire typical of the delayed-type inflammatory T-helper 1 (Th1) CD4$^+$ subset. In contrast, cells taken after clearance of a secondary infection produced IL-4 and acted as effective helper cells for anti-malarial antibody (Ab) synthesis *in vitro*, and thereby had the characteristics of Th2 cells. The appearance *in vivo* of Th1 and then Th2 clones specific for *P. chabaudi*-parasitized erythrocytes (pRBC) supports the proposal from limiting culture analyses that for this malaria parasite resolution of primary parasitaemia is predominantly through the action of cytokines rather than Ab, and that final clearance requires helper cells and specific immunoglobulin.

INTRODUCTION

With the development of techniques to clone and propagate functionally active and antigen (Ag)-specific T lymphocytes *in vitro*,[1] their heterogeneity has become increasingly apparent. Murine CD4$^+$ T-cell clones can be divided into two usually non-overlapping subsets, based on patterns of lymphokine secretion.[2] One subset, Th1, secretes interleukin-2 (IL-2), interferon-gamma (IFN-γ) and lymphotoxin, whilst a second, Th2, produces IL-4, IL-5 and IL-6. Fundamentally different functions are attributed to this differing lymphokine synthesis. Most important with respect to infectious disease, Th1 cells mediate delayed-type hypersensitivity and Th2 cells act as helper cells for the production of specific antibody (Ab) of IgE and IgG1 isotypes.

Abbreviations: Ab, antibody; Ag, antigen; APC, antigen-presenting cells; FITC, fluorescein isothiocyanate; IFAT, indirect fluorescent antibody test; Ig, immunoglobulin; IFN, interferon; IL, interleukin; mAb, monoclonal antibody; MHC, major histocompatability complex; PBS, phosphate-buffered saline; pRBC, parasitized red blood cell; RBC, red blood cell; SN, supernatant; Th, T helper; WEP, Wellcome Experimental Parasitology.

Correspondence: Dr A. W. Taylor-Robinson, Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden Road, Glasgow G61 1QH, U.K.

In malaria, characterization of protective CD4-bearing cells responsive to parasite Ag may facilitate anti-malarial vaccine development as it will provide a means of selecting Ag which induce appropriate effector functions of the CD4$^+$ lymphocyte. That CD4$^+$ cells play a role in protective immunity to *Plasmodium chabaudi* was indicated by bulk culture and limiting dilution analyses of splenic lymphocytes from mice during infection,[3,4] and has been shown more directly by depleting mice of CD4$^+$ and CD8$^+$ T cells before infection with the parasite.[5,6] Limiting dilution analysis revealed a functional heterogeneity of the CD4$^+$ cell precursor frequency following infection and provided indirect evidence that effector mechanisms controlling the acute primary parasitaemia may be predominantly Ab-independent but are superseded by Ab-mediated immunity. To explore this possibility by a different approach, we have established CD4$^+$ clones which react to *P. chabaudi*, from mice recovering from primary parasitaemia, on Day 16, and from mice after a secondary infection. This study describes the characterization of each of these clones *in vitro*, with respect to lymphokine secretion and generation of specific secondary Ab responses. The findings confirm the dual nature of the host immune response to this malaria parasite, indicating that distinct protective functions are operative in early and late stages of primary infection and after reinfection. The protective capacity of each clone has been established *in vivo* and will be

described in a forthcoming communication (A. W. Taylor-Robinson and R. S. Phillips, manuscript in preparation).

MATERIALS AND METHODS

Mice and parasites

Inbred female NIH mice aged 8–12 weeks were bred inhouse. Inbred and congenic mouse strains for the major histocompatability complex (MHC) restriction analysis were purchased from Harlan Olac Ltd, Bicester, Oxon, U.K. A cloned line of the AS strain of *P. chabaudi* subspecies *chabaudi*, obtained originally from Professor D. Walliker (University of Edinburgh, U.K.), was stored in liquid N_2 until used. In immunocompetent mice, a primary infection lasts up to two months and consists of an acute primary parasitaemia lasting 14–18 days (after inoculation of 1×10^5 pRBC) and a period of subpatency followed by usually one more patent parasitaemia (recrudescence). Further infections are resolved more rapidly.

Preparation of parasitized erythrocyte antigens

A crude homogenate of *P. chabaudi* was prepared by freeze-thawing trophozoite/schizont-infected RBC,[7] which were obtained by growing up late ring stages *in vitro*.[8] The lysate was centrifuged (10,000 ***g***, 30 min) and supernatant (SN) stored at −70°. A control lysate of normal RBC from uninfected NIH mice was also prepared. Total protein concentration was determined by a standard bicinchoninic acid assay.[9] Preliminary investigation showed that 200 μg/ml pRBC lysate SN was the optimal concentration of Ag that induced T-cell proliferation and this was used in all *in vitro* stimulations described.

Preparation of lymphocytes and antigen-presenting cells (APC)

Donor NIH mice were either (i) infected with 1×10^5 pRBC and killed on Day 16 of the primary infection (when the acute parasitaemia was in decline), or (ii) infected successively with 1×10^5 and 1×10^7 pRBC 2 months apart and killed 1 week later when the peripheral blood parasitaemia had cleared. Single-cell suspensions of responder cells were prepared from the spleens of mice primed to *P. chabaudi* into RPMI-1640 (Flow Laboratories, Irvine, Ayshire, U.K.) containing 5% foetal calf serum (FCS; Gibco, Paisley, Renfrewshire, U.K.). RBC were lysed using 0·17 M Tris-buffered NH_4Cl, adherent cells removed by glass-wool filtration and enriched T cells collected through nylon-wool[10] into RPMI plus 10% FCS (RPMI/10). Control lymphocyte suspensions enriched for either B cells or T cells were likewise prepared from uninfected mouse spleens (>90% and >85% purity in an indirect fluorescent Ab test, IFAT, respectively).

Antigen-presenting cells (APC) were obtained from spleens of naive syngeneic mice. Cell suspensions at an appropriate concentration in RPMI/10 were irradiated with 30 Gy (3 krads) of gamma radiation from a ^{60}Co source.

Generation and expansion of antigen-reactive T-cell clones

The method for establishing T-cell lines was a modification of that described by Riedlinger *et al.*[11] Cultures were initiated with enriched splenic T cells at 4×10^6/ml, then viable cells subcultured at 1×10^6/ml and 1×10^5/ml, and at each subculture thereafter diluted to a starting concentration of $2{\cdot}5 \times 10^4$/ml. Cells were incubated with APC (1×10^6/ml) in 40 ml RPMI/10 plus pRBC lysate SN in 75-ml culture flasks for 12–15 days (37°, 5% CO_2). One line derived from each *in vivo* priming procedure was cloned by limiting dilution. Anti-CD3 monoclonal antibody (mAb) secreted by the 145-2C11 hybridoma (a gift of Dr R. K. Grencis, University of Manchester, U.K.) stimulated clonal proliferation *in vitro*[12] (used as a 6-day culture SN at 20% v/v unless stated). APC (4×10^6/ml) in RPMI/10 plus anti-CD3 SN and pRBC Ag were plated out at 100 μl/well in 96-well microtitre plates (Nunc, Roskilde, Denmark). Washed cells were diluted in RPMI/10 plus anti-CD3 SN from 500 cells/ml to a probable 1 cell/ml (50–0·1 cells/well) and 100 μl added to APC and Ag. After 7 days incubation (37°, 5% CO_2), 25 μl anti-CD3 (100% v/v SN) were added to all wells. From Day 10, plates were scored for growing clones. APC (4×10^6/ml) were prepared in RPMI/10 containing anti-CD3 mAb SN and Ag and plated out at 1·5 ml/well in a 24-well plate (Linbro, Flow), and clones transferred and incubated for 6 days, when 0·5 ml anti-CD3 SN was added to each well. Ten to 12 days later, confluent clones were re-stimulated with APC and Ag (details as above) and transferred to 10-ml cultures (2×10^6/ml) in 25-ml flasks. The surface phenotype and proliferative response to Ag of each clone was tested and stocks preserved in liquid N_2.

Table 1. MHC restriction analysis of T-cell proliferation to *P. chabaudi*: strains and H-2 haplotypes of mice used for APC (after ref. 33)

		H-2 locus allele					
Strain	Haplotype	K	A_β	A_α	E_β	E_α	D
Classical							
NIH	q	q	q	q	q	q	q
B10.G	q	q	q	q	q	q	q
C57BL/10	b	b	b	b	b	b	b
B10.D2	d	d	d	d	d	d	d
B10.BR	k	k	k	k	k	k	k
B10.AKM	m	m	m	m	m	m	m
B10.R111	r	r	r	r	r	r	r
B10.S	s	s	s	s	s	s	s
Recombinant							
B10.AQR	y1	q	k	k	k	k	d
B10.T(6R)	y2	q	q	q	q	q	d

Surface phenotyping by IFAT

T-cell clones (2×10^5/ml) were tested with rat anti-mouse IgG2b mAb specific for Thy-1.2, CD4, CD8 or CD32 (Fc IgG2b receptor) (Sera-Lab, Crawley Down, Sussex, U.K.). Naive unfractionated spleen cells and normal rat serum acted as the respective controls. Serial doubling dilutions in PBS of each mAb (initial dilution 1:10, 50 μl) were added to 50 μl test suspension in a 96-well plate and incubated for 1 hr at 4°. Cells were incubated (30 min at 37°) with a 1:200 dilution of FITC-conjugated goat anti-rat IgG whole molecule (Sigma, Poole, Dorset, U.K.), washed and resuspended to 0·5 ml in PBS. The percentage of positive cells was determined by fluorescent microscopy.

Lymphocyte proliferation assay

APC (4×10^6/ml) and washed responder cells (2×10^5/ml) were plated out (100 μl/well in RPMI/10) in a 96-well flat-bottomed

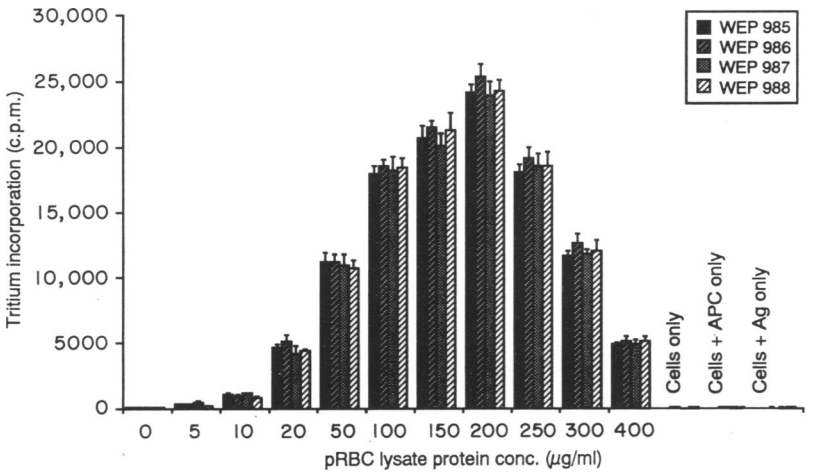

Figure 1. Proliferation of T-cell clones WEP 985–988 to *P. chabaudi in vitro* upon presentation by syngeneic APC.

plate, pRBC and normal RBC lysate SN dilutions (5–400 μg/ml) added (100 μl/well), and the plate incubated for 72 hr (37°, 5% CO_2). Each well received 1·0 μCi (18·5 KBq) [^{3}H]thymidine (Amersham Int., Amersham, Bucks, U.K.) in 20 μl medium for the final 18 hr and radioactive incorporation was measured by liquid scintillation counting. Results are expressed as mean c.p.m. values ± SD for quadruplicate wells. Cells were considered to have given Ag-specific proliferation if values were ≥3 SD more than for appropriate negative controls.

To examine whether presentation of plasmodial Ag to the T-cell clones (H-2^q haplotype) is MHC-restricted, proliferation assays were performed with allogeneic APC. All congenic mice had the B10 genetic background (Table 1); thus, any difference in proliferative response to pRBC Ag presented by APC of varying haplotype was considered to be due to H-2 control.

Generation of lymphokine-containing SN

Clones (4×10^5/ml), cultured with malarial Ag for 6–8 days, were stimulated further with pRBC (or uninfected RBC) Ag (200 μg/ml, 100 μl/well) and APC (4×10^6/ml) in 96-well plates. Pooled SN from quadruplicate wells after 24 hr incubation (37°, 5% CO_2) were stored at −20° until tested. SN from cultured splenic T cells (>86% pure by IFAT) from mice either uninfected or recently recovered from a primary infection (75 days after infection) were controls for all lymphokine assays.

Bioassay of IL-2 and IL-4

CTLL.2 cells[13] were maintained in RPMI/10 plus 10% concanavalin A-stimulated rat spleen cell SN as a source of IL-2. These cells respond to both IL-2 and IL-4, so proliferation was distinguished by performing paired assays either with or without a rat anti-IL-4 mAb (obtained from 11B11 cell SN[14] and purified by affinity chromatography on protein A–sepharose) (Pharmacia, Uppsala, Sweden). Fifty microlitres of mAb (1/20 v/v optimal to neutralize IL-4 activity) or medium alone were added to SN in microtitre wells and incubated for 30 min (37°, 5% CO_2). One hundred microlitres of washed CTLL.2 cells (2×10^4/ml) were added and incubated for 24 hr, when 20 μl [^{3}H]thymidine (1·0 μCi, 18·5 KBq/well) were added for the final 20 hr of culture before determining radioactive uptake by liquid scintillation counting. rMuIL-2 (Genzyme, Boston, MA) standardized CTLL.2 cell proliferation. Results are expressed as mean c.p.m. values for four replicate wells.

Bioassay of IFN-γ

IFN-γ content of SN samples was assayed by inhibition of Semliki Forest virus-directed RNA synthesis in L-929 fibroblasts, as described previously.[15] Serial dilutions of SN were added to infected cultures treated with actinomycin D, and the extent of viral RNA synthesis determined by measuring the amount of [^{3}H]uridine converted into a trichloroacetic acid-insoluble form. Mean c.p.m. values for four replicate cultures were calibrated against $\log_{10}$ IFN-γ dilution using rMuIFN-γ (Research Resources Branch, NIH, Bethesda, MD) as an assay standard. Inhibition of virus multiplication was taken to be directly proportional to IFN-γ titre.

Helper T-lymphocyte function

The ability of each T-cell clone to induce an Ab response to *P. chabaudi in vitro* was assayed by modification of a previously described method.[16] Eight days after stimulation, T cells (4×10^5/ml), APC (4×10^6/ml) and pRBC Ag (200 μg/ml) in RPMI/10 were added to spleen cells (4×10^5/ml) enriched for B cells by nylon-wool filtration (>90% by IFAT). Primed B cells were taken from mice 75 days after being infected (preliminary investigation had shown these to secrete optimal levels of specific Ab). Ten-millilitre volumes were incubated in 25-ml flasks for 9 days (37°, 5% CO_2) and then SN aspirated. Control cultures were set up with naive or post-infection (Day 75) T cells (>87% pure by IFAT). Each SN was concentrated threefold by ultrafiltration at a 10,000 MW cut-off (Amicon, Beverley, MA), dialysed against PBS and assayed for parasite-specific Ab by IFAT[17] using trophozoite/schizont-infected RBC as the target Ag. Results are expressed as the reciprocal Ab titre.

RESULTS

Generation of helper T-cell clones

A total of eight clones (four from each priming procedure), obtained by diluting each parent cell line to 0·5 or 0·1 cell/well, was characterized; they were designated WEP 996–999 (Day 16, primary infection) and WEP 985–988 (post-secondary infec-

Table 2. Proliferation of the T-cell clone WEP 988 *in vitro* upon presentation of 200 μg/ml pRBC lysate Ag by APC of varying haplotype

Mouse strain	Tritium incorporation (c.p.m. ± SD)
NIH	26,314 ± 1369
B10.G	23,488 ± 2140
B10.T(6R)	24,533 ± 1875
C57BL/10	1530 ± 269
B10.D2	1948 ± 596
B10.BR	1620 ± 895
B10.AKM	2074 ± 752
B10.R111	1621 ± 125
B10.S	1621 ± 360
B10.AQR	1328 ± 420

Table 3. IL-2 and IL-4 production by *P. chabaudi*-reactive T-cell clones in the absence and presence of antibody to IL-4

Cell supernatant	CTLL.2 cell proliferation in the absence of anti-IL-4 mAb (c.p.m. ± SD)	CTLL.2 cell proliferation in the presence of anti-IL-4 mAb (c.p.m. ± SD)
rMuIL-2	29,471 ± 2048	28,830 ± 1822
WEP 996	18,412 ± 1738	17,839 ± 1520
WEP 997	24,079 ± 1326	24,100 ± 1658
WEP 998	23,110 ± 1062	22,060 ± 945
WEP 999	23,314 ± 870	22,678 ± 1184
WEP 985	1594 ± 164	411 ± 33
WEP 986	1217 ± 103	394 ± 15
WEP 987	1339 ± 228	383 ± 20
WEP 988	1478 ± 144	417 ± 46
Post-infection splenic T cells	7821 ± 627	6214 ± 439
Naive splenic T cells	4108 ± 330	3891 ± 285
Conditioned medium	380 ± 74	371 ± 32

24-hr SN from cells cultured with syngeneic APC and pRBC Ag were assayed for induction of CTLL-2 cell proliferation.

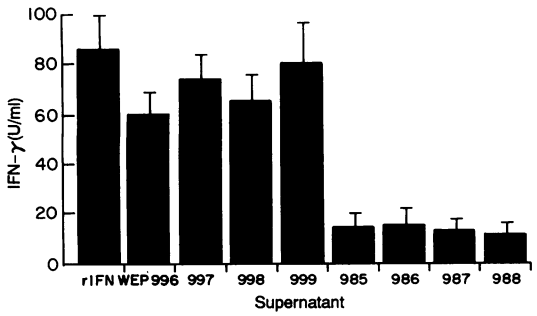

Figure 2. IFN-γ production by *P. chabaudi*-reactive T-cell clones. Twenty-four-hour SN from cells cultured with syngeneic APC and pRBC Ag were assayed for inhibition of viral nucleic acid synthesis.

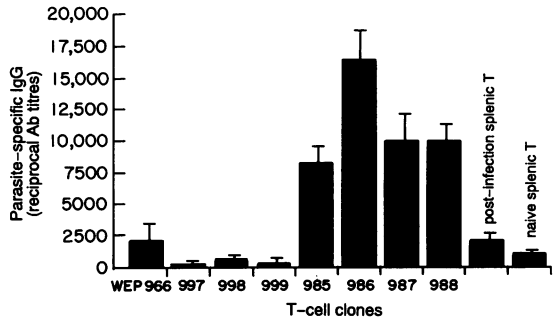

Figure 3. Induction of secondary antibody responses by *P. chabaudi*-reactive T-cell clones. Nine-day SN from cells cultured with pRBC Ag and post-infection B cells were assayed for anti-parasite IgG by IFAT.

tion). Each was homogeneous by IFAT for expression of Thy-1.2 and CD4 Ag.

MHC restriction of *P. chabaudi*-responsive proliferation

Proliferation of clones was similar in terms of both specificity and magnitude (shown for WEP 985–988 in Fig. 1). Responsiveness was dependent upon APC availability and was specific for pRBC, as stimulation with normal RBC gave significantly less proliferation ($P < 0{\cdot}01$) (data not shown). Suppression occurred at high Ag doses, which may have been due to blocking of cell division following repeated exposure of T cells to Ag–APC complexes.[18]

Clones were MHC-restricted in recognition of *P. chabaudi*, proliferating strongly to Ag when presented by H-2-matched APC (NIH, B10.G strains) but not when presented by H-2-disparate APC. All the clones responded similarly and representative data for one clone, WEP 988, are given in Table 2. For the recombinant haplotype B10.T(6R), which has the d allele at the H-2 D locus but carries q alleles at other H-2 loci, APC supported proliferation of similar magnitude to that supported by syngeneic APC. However, with APC from B10.AQR mice, for which only the K locus is occupied by q alleles, each clone failed to proliferate. Thus, not only is responsiveness of the clones MHC restricted, but the restriction elements reside within the class II region.

IL-2 and IL-4 secretion

Culture SN from the eight clones differed substantially in their ability to support CTLL.2 cell proliferation (Table 3). Those clones derived from Day 16 of primary infection, WEP 996–999, provided significantly more support (22,229 ± 2579 c.p.m.) than those clones derived following a secondary infection, WEP 985–988 (1467 ± 218 c.p.m.; $P < 0{\cdot}01$). Although these latter SN gave less support than those from naive (4108 ± 330 c.p.m.) or post-infection (7821 ± 627 c.p.m.) splenic T cells ($P < 0{\cdot}01$), proliferation was significantly raised ($P < 0{\cdot}05$) compared to CTLL.2 cells incubated alone (380 ± 74 c.p.m.). WEP 996–999 SN supported similar proliferation in the presence of anti-IL-4 mAb (21,663 ± 2698 c.p.m.) as in its absence ($P > 0{\cdot}05$). This support, though considerable, was suboptimal compared to that for a rMuIL-2-positive control (28,830 ± 1822 c.p.m.), thereby excluding the possibility that any potential decrement in CTLL.2 growth in the presence of the IL-4-blocking mAb may

not have been detected. Thus, it was concluded that these clones secreted IL-2 but not IL-4 upon antigenic stimulation *in vitro*. For WEP 985–988 SN, target cell growth in the absence of anti-IL-4 mAb was significantly greater than in its presence (401 ± 34 c.p.m.) ($P < 0.01$). Abrogation of low grade CTLL.2 growth by anti-IL-4 mAb indicated secretion of IL-4 but not IL-2 by these clones.

IFN-γ secretion

Profoundly different IFN-γ levels were detected in clonal culture SN (Fig. 2). SN from the Day 16 clones, WEP 996–999, showed significant IFN-γ activity compared to SN controls from post-infection (26·4 ± 3·2 U/ml) and naive (18·7 ± 3·8 U/ml) splenic T cells ($P < 0.01$). In contrast, SN from post-secondary infection clones, WEP 985–988, contained minimal levels of IFN-γ, as counts were not significantly raised compared with those for the conditioned medium negative control (14·1 ± 1·7 U/ml; $P > 0.05$). For WEP 996–999 SN, higher titres were attained with pRBC Ag than with uninfected RBC Ag ($P < 0.01$) (data not shown).

Helper T-cell activity

Clones derived following two successive infections, WEP 985–988, induced specific Ab responses *in vitro* (Fig. 3), which were significantly greater than those induced by the Day 16 clones, WEP 996–999 ($P < 0.01$). Incubation of these latter cells with post-infection B cells and pRBC Ag gave IFAT Ab titres as low as those for naive splenic T cells, and, WEP 996 apart, significantly lower than for similarly cultured post-infection T cells ($P < 0.05$).

DISCUSSION

The existence of two major functionally distinct Th cell subsets is now established in mice and indicated in humans.[19,20] In view of this, we have examined whether the distinct biphasic immune response to *P. chabaudi in vivo*[17] may be explained by the presence of different CD4+ T-cell subsets during primary and subsequent infections. The functional heterogeneity of the CD4+ T-cell response to *P. chabaudi* has been evaluated previously by application of cytokine assays to a limiting dilution system on whole lymphocyte populations.[4] This work indicated that during the primary parasitaemia of a *P. chabaudi* infection, the predominant precursor T-cell response was that of the Th1-type. Thereafter, the frequency of these cells declined, whilst that of Th2-type cells increased. Through analysing the precursor frequencies of parasite-specific T cells, it was further shown that there was an association between the varying frequencies of Th1 and Th2 CD4+ cells in the host during a *P. chabaudi* infection and the resolution of that infection. However, it was not demonstrated directly that these cells were protective. To address this issue, we have raised CD4+ T-cell lines to *P. chabaudi in vitro* using responder cells taken from the spleens of infected mice at a time corresponding to the remission of the acute parasitaemia (on Day 16 of primary infection) and following clearance of a secondary infection. By cloning these lines by limiting dilution, we obtained a panel of CD4+ clones which could be subjected to a variety of assays to detect cytokine production and helper activity for malaria-specific Ab synthesis. In addition, all of these clones could reconstitute protection to CD4-depleted mice infected with *P. chabaudi* (A. W. Taylor-Robinson and R. S. Phillips, manuscript in preparation).

All eight CD4+ clones analysed proliferated *in vitro* to *P. chabaudi* pRBC Ag presented in the context of one or more molecules encoded within the I-A or I-E subregions of the H-2 complex; this has also been reported for a *P. chabaudi adami*-reactive CD4+ clone.[21] A clear distinction could be made between clones that had mutually exclusive cytokine secretion patterns of IL-2 plus IFN-γ, or IL-4, i.e. Th1 and Th2 types.[2] It was found that the four clones with the Th1 pattern of cytokine secretion were those derived from the primary infection (WEP 996–999). In contrast, the four clones derived from donor mice recovered from a secondary infection (WEP 985–988) were all of the Th2 type. These results extend the work of Brake *et al.*[21] who characterized a *P. chabaudi adami*-reactive CD4+ clone which secreted IL-2 and IFN-γ, indicative of the Th1 subset. Our present report is the first to describe the successful *in vitro* propagation of malaria-specific Th2 CD4+ clones.

Regarding helper T-cell activity, clones primed by two complete infections (WEP 985–988) induced significant Ab titres *in vitro*, whereas those exposed to *P. chabaudi* for 16 days of primary infection (WEP 996–999) induced insignificant titres. The latter is also true of an uncloned CD4+ line propagated *in vitro* from splenic T cells taken 20 days after the start of primary infection (A. W. Taylor-Robinson and R. S. Phillips, unpublished observations). These results are consistent with the demonstration that the majority of CD4+ cells elicited during the acute phase of *P. chabaudi* infection provide poor help for Ab synthesis, whilst good helpers increase in frequency later in primary infection.[4] Moreover, as it was the Th2 clones that acted in a helper capacity for specific Ab production *in vitro* and the Th1 clones that did not do so, there was thus a correlation between the lymphokines secreted by each *P. chabaudi*-reactive CD4+ clone and its putative effector function, as would be predicted. IL-4 induces Ab isotype switching from IgM to IgG1, the synthesis of which by B cells is then up-regulated.[22] IgG1 is the Ig isotype that is present in peripheral blood serum around 3–4 weeks after initiation of infection, and in immune mice,[23] and is very likely that which is detected as specific Ab by IFAT.[17] This is at a time when the predominant CD4+ T-cell response is that of a Th2 cell (this publication and ref. 4).

As IFN-γ is an antagonist of IL-4 activity on B cells,[24,25] this can explain the inability of the Th1 clones to help mount an anti-malarial Ab (presumably IgG1) response. At the time when the clones were taken, 16 days after the start of infection (when the acute parasitaemia was in decline), the dominant CD4+ cell response was that of the Th1 subset;[4] this response would include the secretion of IFN-γ as shown here. This would explain the low levels of IgG1 Ab (both specific and non-specific) which are produced during the primary parasitaemia.[23,26] It would also explain the predominant IgG2a Ab response to *P. chabaudi* during this time; IFN-γ is a switch factor for the production by B cells of this Ig isotype.[27] There is no evidence at present for IgG2a being involved in the protective Ab response, which correlates with the low titre of *P. chabaudi*-specific Ab in the peripheral blood during the acute parasitaemia.[17] This may be accounted for by the very rapid removal from circulation of any IgG2a, by binding either to the abundant pRBC present under a heavy parasite burden at this time of infection, or to activated macrophages. In this latter case, it is

thought that macrophage expression of the Fcγ receptor is increased under IFN-γ regulation,[28] thereby up-regulating the binding of the increased amounts of the IgG2a, the production of which is promoted by IFN-γ.

In *P. chabaudi*-infected C57BL/6 mice, IFN-γ secretion is maximal just before the peak of primary parasitaemia, then decreases to negligible levels by Day 25.[29,30] Similar findings here showed, at the clonal level, that CD4+ cells derived from the first wave of primary infection secreted considerable amounts of IFN-γ *in vitro*, whereas those primed to *P. chabaudi* through infection and one or two reinfections produced negligible quantities of this cytokine; an uncloned CD4+ line raised to *P. chabaudi* following three infections confirmed the results for the post-secondary infection clones (A. W. Taylor-Robinson and R. S. Phillips, unpublished observations). Evidence therefore implicates IFN-γ in the reduction of acute parasitaemia to low or subpatent levels. This is presumably through the activation of macrophages and the subsequent release of toxic mediators such as tumour necrosis factor, reactive oxygen intermediates and nitrogen oxide metabolites.[31] As IFN-γ and IL-4 are secreted by Th1 and Th2 cells, respectively, the relative contributions of IFN-γ-dependent and IL-4-dependent pathways in immunity to *P. chabaudi* infection presumably reflect the balance between the proportions of the two subsets of CD4+ cells at any given time. The data described here for CD4+ clones characterized *in vitro* add support to this concept to that available at the lymphocyte population level.[4] In this light, it is clear that the clones analysed in this study were not atypical of the predominant CD4+ cell type present in the host spleen at those times during infection when the donor spleens were taken. Furthermore, each of these clones is now known to be capable of conferring protection upon adoptive transfer to T-cell-depleted mice infected with *P. chabaudi* (A. W. Taylor-Robinson and R. S. Phillips, manuscript in preparation). Therefore, the results described here add weight to the proposal that protective immunity to *P. chabaudi* evolves during infection from a predominantly non-Ab to a predominantly Ab-mediated mechanism.[32] In addition, they also have potentially important implications for malaria vaccine design, as it is shown at the clonal level that both Th1 and Th2 cells are involved in immunity to asexual malaria parasites. Hence, protective T-cell epitopes recognized either exclusively by one subset or similarly by both subsets should be considered in future as vaccine candidates.

Overall, it would appear that the divergent reactivities of CD4+ populations during *P. chabaudi* infections show a change in the mechanism of protection with time and indicate that this switch is dependent upon whether one or other CD4+ subset is predominant at a given time following infection. A similar mechanism involving a strong Th2 response without Th1 activation, or vice versa, provides a plausible explanation of immune responses to infection with two other rodent plasmodia, *P. yoelii* 17X and *P. chabaudi adami*, respectively. These represent extremes in the repertoire of protective immunity to malaria, one predominantly humoral and the other cell-mediated.

ACKNOWLEDGMENTS

A. W. Taylor-Robinson was the recipient of a Medical Research Council research training scholarship. We thank Richard Grencis and Peter Wood (University of Manchester) for advice about T cell cloning and IFN-γ assays. The MHC restriction analysis was performed through a Wellcome Trust grant to Malcolm Kennedy (WLEP). David McLaughlin provided excellent technical assistance.

REFERENCES

1. Fathman C.G. & Kimoto M. (1981) Studies utilizing murine T cell clones: Ir genes, Ia antigens and MLR stimulating determinants. *Immunol. Rev.* **54,** 57.
2. Mosmann T.R. & Coffman R.L. (1987) Two types of mouse helper T-cell clone: implications for immune regulation. *Immunol. Today*, **8,** 223.
3. Langhorne J. & Simon B. (1989) Limiting dilution analysis of the T cell response to *Plasmodium chabaudi chabaudi* in mice. *Parasite Immunol.* **11,** 545.
4. Langhorne J., Gillard S., Simon B., Slade S. & Eichmann K. (1989) Frequencies of distinct response kinetics for cells with T_h1 and T_h2 characteristics during infection. *Int. Immunol.* **1,** 416.
5. Süss G., Eichmann K., Kury E., Linke A. & Langhorne J. (1988) Roles of CD4- and CD8-bearing T lymphocytes in the immune response to the erythrocytic stages of *Plasmodium chabaudi. Infect. Immun.* **56,** 3081.
6. Langhorne J., Meding S.J., Eichmann K. & Gillard S.S. (1989) The response of CD4+ T cells to *Plasmodium chabaudi chabaudi. Immunol. Rev.* **112,** 71.
7. McDonald V. & Sherman I.W. (1980) *Plasmodium chabaudi*: humoral and cell-mediated responses of immunized mice. *Exp. Parasitol.* **49,** 442.
8. Trager W. & Jensen J.B. (1976) Human malaria parasites in continuous culture. *Science*, **193,** 673.
9. Smith P.K., Krohn R.I., Hermanson G.T., Mallia A.K., Gartner F.H., Provenzano M.D., Fujimoto E.K., Goeke N.M., Olson B.J. & Klenk D.C. (1985) Measurement of protein using bicinchoninic acid. *Analyt. Biochem.* **150,** 76.
10. Julius M.H., Simpson E. & Herzenberg L.A. (1973) A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3,** 645.
11. Riedlinger J., Grencis R.K. & Wakelin D. (1986) Antigen-specific T-cell lines transfer protective immunity against *Trichinella spiralis in vivo. Immunology*, **58,** 57.
12. Leo O., Foo M., Sachs D.H., Samelson L.E. & Bluestone J.A. (1987) Identification of a monoclonal antibody specific for a murine T3 polypeptide. *Proc. natl. Acad. Sci. U.S.A.* **84,** 1374.
13. Gillis S. & Smith K.A. (1977) Long term culture of tumour-specific cytotoxic T cells. *Nature (Lond.)*, **268,** 154.
14. Ohara J. & Paul W.E. (1985) Production of a monoclonal antibody to and molecular characterisation of B-cell stimulatory factor-1. *Nature (Lond.)*, **315,** 333.
15. Allen P.T. & Giron D.J. (1970) Rapid sensitive assay for interferons based on the inhibition of MM virus nucleic acid synthesis. *Appl. Microbiol.* **20,** 317.
16. Pearson C.D., McLean S.A., Tetley K. & Phillips R.S. (1983) Induction of secondary antibody responses to *Plasmodium chabaudi in vitro. Clin. exp. Immunol.* **52,** 121.
17. McLean S.A., Pearson C.D. & Phillips R.S. (1982) *Plasmodium chabaudi*: relationship between the occurrence of recrudescent parasitaemias in mice and the effective levels of acquired immunity. *Exp. Parasitol.* **54,** 213.
18. Suzuki G., Kawase Y., Koyasu S., Yahara I., Kobayashi Y. & Schwartz R.H. (1988) Antigen-induced suppression of the proliferative response of T cell clones. *J. Immunol.* **140,** 1359.
19. Mosmann T.R. & Coffman R.L. (1989) T_H1 and T_H2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* **7,** 145.
20. Romagnani S. (1991) Human T_H1 and T_H2 subsets: doubts no more. *Immunol. Today*, **12,** 256.

21. Brake D.A., Long C.A. & Weidanz W.P. (1988) Adoptive protection against *Plasmodium chabaudi adami* malaria in athymic nude mice by a cloned T cell line. *J. Immunol.* **140,** 1989.
22. Isakson P.C., Pure E., Vitetta E.S. & Krammer P.H. (1982) T cell-derived B cell differentiation factor(s). Effect on the isotype switch of murine B cells. *J. exp. Med.* **155,** 734.
23. Langhorne, J. Evans C.B., Asofsky R. & Taylor D.W. (1984) Immunoglobulin isotype distribution of malaria-specific antibodies produced during infection with *Plasmodium chabaudi adami* and *Plasmodium yoelii*. *Cell. Immunol.* **87,** 452.
24. Coffman R.L. & Carty J. (1986) A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-γ. *J. Immunol.* **136,** 949.
25. Rabin E.M., Mond J.J., Ohara J. & Paul W.E. (1986) Interferon-γ inhibits the action of B cell stimulatory factor (BSF)-1 on resting B cells. *J. Immunol.* **137,** 1573.
26. Falanga P.B., D'Imperio Lima M.R., Coutinho A. & Pereira Da Silva L. (1987) Isotypic pattern of the polyclonal B cell response during primary infection by *Plasmodium chabaudi* and in immune-protected mice. *Eur. J. Immunol.* **17,** 599.
27. Snapper C.M. & Paul W.E. (1987) Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science*, **236,** 944.
28. Warren M.K. & Vogel S.N. (1985) Opposing effects of glucocorticoids on interferon-gamma-induced murine macrophage Fc receptor and Ia antigen expression. *J. Immunol.* **134,** 2462.
29. Slade S.J. & Langhorne J. (1989) Production of interferon-gamma during infection of mice with *Plasmodium chabaudi chabaudi*. *Immunobiology*, **179,** 353.
30. Stevenson M.M., Fong Tam M., Belosevic M., Van Der Meide P.H. & Podoba J.E. (1990) Role of endogenous gamma interferon in host response to infection with blood-stage *Plasmodium chabaudi* AS. *Infect. Immun.* **58,** 3225.
31. Melancon-Kaplan J. & Weidanz W.P. (1990) Role of cell-mediated immunity in resistance to malaria. In: *Malaria. Host Responses to Infection* (ed. M. M. Stevenson), p. 37. CRC Press, Boca Raton.
32. Langhorne J. (1989) The role of CD4+ T cells in the immune response to *Plasmodium chabaudi*. *Parasitol. Today*, **5,** 362.
33. Klein J. (1986) *Natural History of the Major Histocompatability Complex*. John Wiley & Sons Inc., New York.