Immunology 1991 **73** 327–333

ADONIS 001928059100160J

Patterns of cytokine production and proliferation by T lymphocytes differ in mice vaccinated or infected with *Schistosoma mansoni*

R. M. PEMBERTON, L. E. SMYTHIES, A. P. MOUNTFORD & R. A. WILSON
Department of Biology, University of York, York

Accepted for publication 25 March 1991

SUMMARY

C57BL/6 mice vaccinated with irradiated cercariae of *Schistosoma mansoni* are highly resistant to challenge infection. To examine the role of T-helper (Th) activity in these vaccinated (V20) mice, cells from skin- and lung-draining lymph nodes (LN) and the spleen were cultured *in vitro* with soluble schistosomular antigen. Peak proliferation and release of T-cell growth factor (TCGF) by axillary LN cells on Day 5, and by mediastinal LN cells on Day 18, reflected the kinetics of parasite migration. High levels of interferon-gamma (IFN-γ) were detected and production was prolonged, particularly in the mediastinal LN. The majority of the above activity was ablated with anti-CD4 antibody. IFN-γ production by spleen cells increased, whilst proliferation and TCGF release remained low. Although levels of proliferation were similar, more IFN-γ was released by LN cells from V20 mice than by those from mice infected with normal parasites (NI). This difference in IFN-γ production was magnified by the greater number of cells in LN of V20 than NI mice. On Day 22 post-exposure, 24-fold more IFN-γ was produced per pair of axillary LN in the former group. LN cells from V20 mice produced interleukin (IL)-2 and IL-4, whereas those from NI mice released IL-2 but negligible IL-4. Greater quantities of IL-3 were secreted by cells from V20 than from NI mice. These results support the conclusion that IFN-γ-producing memory Th cells, generated in the LN of V20 mice, play an important role in protective immunity against *S. mansoni*.

INTRODUCTION

A single percutaneous exposure of mice to optimally irradiated cercariae of *Schistosoma mansoni* induces up to 70% protection against challenge infection.[1] There is much evidence that this resistance is the result of a specific immune response.[2,3] In contrast, resistance to challenge infection in mice previously exposed to normal cercariae correlates with tissue egg count and liver pathology (reviewed in ref. 4) rather than specific immunity. Moreover, single sex infections, which fail to produce eggs, induce little or no resistance (reviewed in ref. 1).

Optimally irradiated parasites show a slower and truncated passage through the host compared with normal parasites,[5] some persisting for longer in the skin-draining lymph nodes (LN) and none migrating further than the lungs.[6] The importance of these LN to the induction of immunity can be gauged from the significant loss of resistance which follows their excision before or up to 20 days after percutaneous vaccination.[7]

Abbreviations: APC, antigen-presenting cells; C, complement; DTH, delayed-type hypersensitivity; IFN-γ interferon-gamma; IL, interleukin; LN, lymph node; PBS, phosphate-buffered saline; RS, concanavalin A-stimulated rat spleen cell supernatant; SSP, soluble 18-hr schistosomular protein; TCGF, T-cell growth factor; Th, T helper.

Correspondence: Dr R. M. Pemberton, Dept. of Biology, University of York, York YO1 5DD, U.K.

Immunity following vaccination with irradiated cercariae is dependent upon the presence of CD4+ T cells[8,9] and is unaffected by depletion of CD8+ T cells.[9] Although the precise role of CD4+ T helper (Th) cells has not been established, interest has focused on their ability to produce interferon-gamma (IFN-γ).[3] P strain mice, which fail to become resistant, are unable to mount a delayed-type hypersensitivity (DTH) reaction and possess T cells that are deficient in IFN-γ production.[10] Conversely, T lymphocytes from protectively vaccinated mice will produce IFN-γ in response to soluble schistosome antigens *in vitro*.[10]

The present study set out to determine whether schistosome-specific T-cell responses in mice differ following protective vaccination compared with a normal infection. We have determined the timing and magnitude of schistosome-specific proliferation of cells from LN draining the skin and lungs, and from spleens, and related this to the previously described differences in parasite migration. In parallel, we have characterized the profile of cytokines produced by these T lymphocytes to establish whether protection is associated predominantly with the induction of a particular Th cell subset. At later times we find elevated production of IFN-γ in vaccinated mice compared with those exposed to normal parasites, which is not accompanied by high IL-2 activity. Relatively low levels of IL-4 have been detected but these are nevertheless higher after vaccination than

normal exposure. This pattern argues against selective generation of Th1 cells in vaccinated animals but in favour of IFN-γ-producing effector T cells.

MATERIALS AND METHODS

Host and parasites

Female C57BL/6 mice, weighing 17–20 g, were bred at the University of York. A Puerto Rican isolate of *Schistosoma mansoni* was maintained by passage through LACA mice and albino *Biomphalaria glabrata* snails.

Vaccination and infection

Mice were anaesthetized with 10% Sagatal (May & Baker) in 10% ethanol (0·01 ml/g body wt) and exposed to 500 cercariae via the shaved abdomen. Two experimental regimes were used: V20, mice vaccinated with cercariae attenuated with 20 krads of gamma radiation from a ^{60}Co source (Department of Radiobiology, Cookridge Hospital, Leeds); NI, mice exposed to normal cercariae.

Preparation of responder lymphocytes and antigen-presenting cells (APC)

Responder lymphocytes were obtained from (i) axillary and (ii) inguinal LN, draining the abdominal vaccination site, (iii) the mediastinal LN, draining the lungs where the majority of V20 parasites die, and (iv) the spleens, of three naive, V20 or NI mice. To obtain single cell suspensions, LN were teased apart with forceps and spleens were pressed through stainless steel mesh. Cells were washed and resuspended in RPMI-1640 (Flow Laboratories, Irvine, Ayrshire, U.K.) containing 10% foetal calf serum (Globepharm Ltd, Esher, Surrey, U.K.), penicillin (200 U/ml), streptomycin (100 μg/ml), 2 mM L-glutamine and 5×10^{-5} M 2-mercaptoethanol (RPMI/10).

APC preparation: naive splenocytes ($1–6 \times 10^7$/ml) were incubated at 37° with 50 μg/ml mitomycin C for 30 min and washed twice. Following a further 30-min incubation without mitomycin and two washes, APC were resuspended in RPMI/10.

Preparation of soluble 18-hr schistosomular protein (SSP)

Cercariae were mechanically transformed *in vitro* as described by Ramalho-Pinto *et al.*[11] Cercarial heads were isolated by centrifugation on a discontinuous density Percoll gradient[12] and washed twice in RPMI/10. Following 18 hr incubation in RPMI/10 at 37°, heads were washed twice and resuspended in phosphate-buffered saline (PBS). Soluble proteins were released by sonication (21 khz at 6·5 μm amplitude) for 1 min and insoluble material was removed by centrifugation for 1 hr at 100,000 ***g***.

Before use in *in vitro* assays, SSP was sterilized by exposure to UV-C light (10 min, 15 cm from a 15W Philips lamp) and stored in aliquots at −80°.

Lymphocyte proliferation and generation of lymphokine-containing supernatants

Responder cells (10^5) and APC (2×10^5), with or without SSP, were cultured for 5 days (37°, 6% CO_2) in 200 μl RPMI/10 in flat-bottomed 96-well microtitre plates. Each well received 18·5 KBq [^{3}H]thymidine (specific activity 185 GBq/mmol, 37 MBq/ml; Amersham International plc, Amersham, Bucks, U.K.) for the final 18 hr and incorporation into cellular DNA was measured by liquid scintillation counting. Results are expressed as mean values (±SEM) for antigen-specific proliferation (d.c.p.m.), i.e. (c.p.m. in presence of SSP) − (mean c.p.m. in absence of SSP) for three or four replicate microwells.

Culture supernatants, pooled from at least four identical wells after 72 hr incubation, were stored at −80° until analysis for lymphokine activity.

Antibodies and cytokines

Anti-Thy-1 monoclonal antibody, YTS 154.7, and baby rabbit complement (C) were purchased from Seralab Ltd, Crawley Down, Sussex, U.K. Monoclonal antibody ascites fluids against lymphocyte surface markers CD4 (YTS 191.1) and CD8 (YTS 169.4) were prepared in nude mice. Serum containing monoclonal rat anti-murine (Mu) IL-2 antibody was obtained from nude mice injected with S4B6 cells[13] and was heat-inactivated before use in neutralization assays. Monoclonal rat anti-MuIL-4 antibody was purified, using an Affi-Gel Protein A MAPS II Kit (Bio-rad Laboratories Ltd, Watford, Herts, U.K.), from ascites fluid obtained from nude mice injected with 11B11 cells.[14] Recombinant (r) MuIL-2 and MuIL-4 were purchased from Genzyme Corporation, Boston, MA and used to standardize S4B6 and 11B11 antibodies. Maximum CTLL.2 cell proliferation (see below) was typically 35,000 c.p.m. with rIL-2 and 10,000 c.p.m. with rIL-4. Neutralization of this proliferation was entirely specific using S4B6 serum at a final concentration of 1/1000 and purified 11B11 at 0·5 μg/ml. IFN-γ-ELISA reagents are described below.

Bioassay of IL-2 and IL-4 in culture supernatants

CTLL.2 cells[15] were maintained in RPMI/10, plus 10% concanavalin A-stimulated rat spleen cell supernatant (RS) as a source of IL-2. For assay of total TCGF activity, 10^4 washed cells in 50 μl were incubated in 96-well round-bottomed microwells with 50 μl of undiluted or diluted test sample for 24 hr. [^{3}H]thymidine (18·5 KBq/well) was added for the final 4 hr and uptake was assessed by liquid scintillation counting. Results are expressed as the mean c.p.m. of three identical wells.

IL-2- and IL-4-driven proliferative responses were distinguished using a method similar to that of Firestein *et al.*[16] Duplicate twofold serial dilutions of supernatant were prepared in 25 μl volumes in round-bottomed microwells. Medium alone, or antibodies to recombinant cytokines IL-2 or IL-4, or both, were then added in 25-μl volumes at the highest allowable final concentrations (v/v) giving specific neutralization of CTLL.2 cell proliferation to rMuIL-2 or rMuIL-4 (see above). After 30 min incubation at 37°, 10^4 CTLL.2 cells were added to a final volume of 100 μl. Proliferation was measured after 24 hr by [^{3}H]thymidine incorporation. Results are expressed as a percentage of the maximum CTLL.2 response obtained with RS.

Bioassay of IL-3

This assay is described elsewhere.[17] Briefly, IL-3-dependent 32-D cells, maintained in RPMI/10 with 20% WEHI-3 conditioned medium, were washed three times and suspended at 2×10^5/ml in serum-free RPMI. 50 μl aliquots (10^4 cells) were added to 50 μl of test supernatant in triplicate round-bottomed microwells and incubated for 24 hr at 37°. Proliferation was measured by [^{3}H]thymidine incorporation during the final 4 hr.

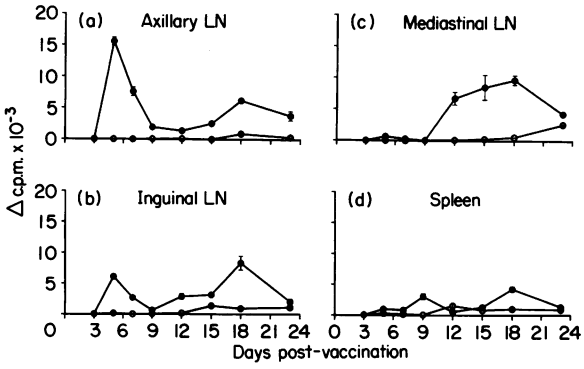

Figure 1. Lymphocyte proliferation of LN or spleen cells from V20 mice. Responder cells (10^5) from vaccinated (●) or naive (○) mice were cultured with APC (2×10^5) for 5 days in the presence or absence of 100 μg/ml SSP. *y*-axis, Δc.p.m. ± SEM for triplicate wells. Background levels in the absence of antigen were always less than 1000 c.p.m. for LN and less than 4000 c.p.m. for spleen cell cultures.

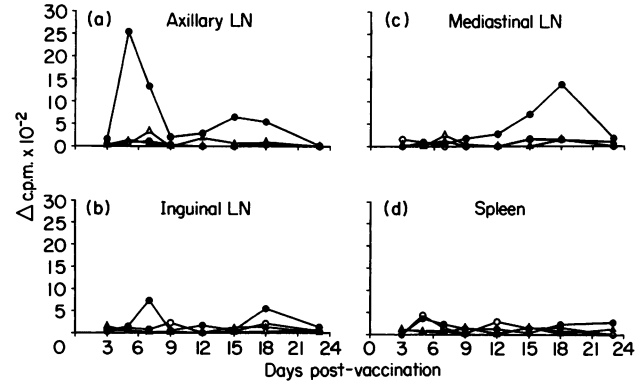

Figure 2. TCGF release by LN or spleen cells from V20 mice. *y*-axis, CTLL.2 cell proliferation in response to 72-hr culture supernatants from vaccinated (circles) or naive (triangles) responder cells cultured with APC in the presence (solid symbols) or absence (open symbols) of 100 μg/ml SSP.

Measurement of IFN-γ in culture supernatants

A modified double antibody sandwich ELISA (described in detail elsewhere; L. E. Smythies *et al.*, manuscript in preparation) was developed to quantify IFN-γ in 72 hr culture supernatants. Briefly, rat anti-IFN-γ monoclonal antibody R4-6A2[18] was used to coat the wells and a polyclonal rabbit anti-MuIFN-γ, R498[19] was used as the detection antibody. rMuIFN-γ obtained from CHO 211A-transfected cells and calibrated in an anti-viral replication assay was used as an assay standard.[20] Results are expressed as mean absorbance values from duplicate microwells.

Statistical analysis

In order to reflect variation in responder lymphocyte populations from well to well, cell proliferation for replicate culture wells is represented as mean c.p.m. ± SEM for triplicates or quadruplicates. Standard errors are not displayed for cytokine assays, since supernatants were obtained from parallel cultures and replicate wells were pooled.

RESULTS

Experiments illustrated in Figs 1, 2 and 3 were performed in parallel cultures using the same cell suspension. The time-course shown is representative of three experiments.

Proliferation of cells to soluble schistosomular antigen following vaccination

Earliest schistosome-specific proliferation was observed by cells from axillary (Fig. 1a) and inguinal (Fig. 1b) LN from V20 mice, with peak responsiveness on Day 5. A second response, of greater magnitude in inguinal than axillary LN (Fig. 1a, b), was observed with a peak between Days 15 and 22. Responses for both sets of LN declined after Day 22 and reached background levels by Day 35 (not shown). Schistosome-specific proliferation by mediastinal LN cells was not detected until at least 10 days after vaccination (Fig. 1c). Maximum proliferation was always coincident with the later peak of proliferation by skin-draining LN cells, i.e. 18–22 days after vaccination. Subsequent decline in proliferation was more gradual than for skin-draining LN, but had reached background levels by Day 35 (not shown). Although antigen-specific proliferation by spleen cells was detected (Fig. 1d), levels were low in comparison with LN responses.

Demonstration of T-cell dependence of LN cell proliferation

Following pre-treatment with anti-Thy.1 antibody (Table 1), axillary and inguinal responses (Day 6 post-vaccination) were inhibited by 95% and 85%, respectively, and the mediastinal LN response (Day 20) was inhibited by 100%, confirming that the majority of proliferation was T-cell-mediated. Addition of supernatants from undepleted cultures failed to reconstitute proliferation in T-cell-depleted cultures (not illustrated), supporting the notion that these assays recorded proliferation of T cells and not of B cells dependent upon TCGF.

Seven days post-vaccination, virtually all proliferation by axillary LN cells could be ascribed to Th cells: inclusion of anti-CD4 antibody during incubation resulted in 90% inhibition at 1/40 and 76% at 1/400; anti-CD8 antibody had no inhibitory effect but increased the response to 109% at 1/40 and 147% at 1/400. A similar result was obtained for inguinal LN cells (Day 7) with anti-CD4. However, 54% inhibition of cell proliferation was detectable in the presence of anti-CD8 antibody at the highest concentration tested (1/40). This inhibition was specific, since TCGF activity remained high.

The majority of proliferation by mediastinal LN cells (Day 22) was inhibited by antibody to CD4 (93% at 1/40; 82% at 1/400; 55% at 1/4000). Less inhibition was obtained with anti-CD8 (81% at 1/40; 48% at 1/400; 15% at 1/4000).

TCGF release by schistosome-specific T cells

Peak levels of TCGF activity released by axillary (Fig. 2a), inguinal (Fig. 2b) and mediastinal (Fig. 2c) LN cells coincided temporally with previously observed proliferative responses. TCGF activity was barely detectable in supernatants from spleen cell cultures (Fig. 2d) and mirrored proliferative responses in being low compared with LN cell cultures.

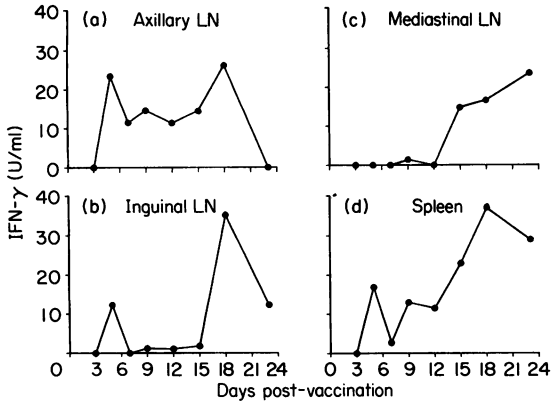

Figure 3. ELISA measurement of IFN-γ release by LN or spleen cells from V20 mice. Culture supernatants were tested 72 hr after incubation of responder cells with APC in the presence of 100 μg/ml SSP. Background levels of IFN-γ release by naive cells or cells from V20 mice cultured in the absence of antigen were less than 7 U/ml.

Release of IFN-γ by LN and spleen cells in response to SSP following vaccination

IFN-γ production by axillary (Fig. 3a) and inguinal (Fig. 3b) LN cells was first detected at Day 5, being followed by a second peak at Day 18, which, in comparison with proliferative responses (cf. Fig. 1.) was disproportionately high. IFN-γ production by mediastinal LN cells (Fig. 3c) was not detected until Day 15 and remained high at Day 23, in contrast to declining proliferation (cf. Fig. 1).

IFN-γ was first detected in spleen cell cultures (Fig. 3d) on Day 5 and increased with time after vaccination to a level at Day 21 which was higher than those seen for LN cell populations.

Phenotype of T cells releasing IFN-γ in V20 mice

72 hr supernatants, from cultures of axillary LN cells taken from V20 mice on Day 7 and incubated in the presence of monoclonal antibodies to CD4 or CD8 (see above), were tested for the presence of IFN-γ. Anti-CD4 antibody gave profound inhibition of IFN-γ release (97% at 1/40; 93% at 1/400), whilst anti-CD8 was less effective (63% at 1/40; 23% at 1/400). Furthermore, at a concentration of 1/4000, where TCGF production was unaffected (data not shown), anti-CD4 inhibited IFN-γ production by 74%, while anti-CD8 gave only 28% inhibition.

Comparison of schistosome-specific T-cell proliferation following exposure to normal or irradiated cercariae

In two separate experiments, the generation of schistosome-specific T cells capable of proliferating *in vitro* to SSP was compared in NI and V20 mice. Supernatants from parallel cultures were assayed for the presence of cytokines (see below). Results (Fig. 4) show that axillary LN responses were of similar magnitude at Days 4 or 7 following exposure to normal or irradiated parasites. Subsequently, there was some indication of a slower decline in the proliferative response in NI than in V20 mice, particularly in Exp. 1.

The two exposure regimes generated mediastinal LN responses (Fig. 4, Exp. 2) of similar magnitude by Day 15. From then until Day 22, there was a more rapid decline in proliferation following normal infection.

IL-2 and IL-4 production by draining LN cells from NI or V20 mice

The profiles of TCGF production by axillary and mediastinal LN cells from V20 and NI mice (not shown) were virtually identical to those of proliferation shown in Fig. 4.

TCGF activity from cultures of V20 axillary LN cells (Day 7 post-exposure) was partially inhibited by antibodies to either IL-2 or IL-4 (Fig. 5a) and was totally neutralized by the combination of antibodies, confirming the presence of both lymphokines. In contrast, TCGF activity from NI cells was totally neutralized by antibody to IL-2, with little inhibition by anti-IL-4 antibody. The inclusion of both antibodies gave no further inhibition from that effected by anti-IL-2 antibody alone, confirming the presence of IL-2 and the absence of IL-4.

A similar pattern was observed for the mediastinal LN (Day 22; Fig. 5b): both IL-2 and IL-4 activities were present in supernatants from V20 cells; only IL-2, and negligible IL-4, was released by NI cells.

Schistosome-specific IFN-γ and IL-3 production following exposure to normal or irradiated parasites

From Day 7 onwards, significantly more IFN-γ was released by cells from V20 than from NI mice (Exps 1 and 2). The differential in IFN-γ production increased with time after exposure and by Day 22 (Exp. 2) there was a ninefold increase in IFN-γ production by cells from V20 mice. The same pattern was produced for mediastinal LN responses (Fig. 6a, Exp. 2), with between three- and sixfold greater IFN-γ production by V20 LN cells from Day 15 onwards. The profile of IL-3 production by V20 and NI axillary LN cells in Exp. 1 (Fig. 6b) was similar between Days 4 and 14. Activity was slightly higher for NI mice at Days 7 and 11. In Exp. 2 (Fig. 6b) more IL-3 was produced by axillary LN cells from V20 mice than NI mice between Days 7 and 22. A similar pattern was observed for the mediastinal LN where, particularly at Days 18 and 22, IL-3 production by NI cells was in decline, but in V20 cultures was increasing (Fig. 6b).

DISCUSSION

We have observed schistosome-specific T-cell proliferation and TCGF production *in vitro* by lymphocytes obtained from LN draining the skin exposure site, the mediastinal LN draining the lungs, and the spleens of V20 mice. The majority of dividing T cells appeared to be $CD4^+$, though a contribution by $CD8^+$ cells is inferred, particularly in the mediastinal LN.

Generation of schistosome-specific Th cell activity in the skin-draining LN on Day 5 after vaccination coincided with the presence of maximum numbers of parasites and levels of parasite-released material in these LN.[6] Subsequent decline in the Th response coincided with reduction in levels of released material by Day 9, despite the continued presence of parasites themselves.[6] The large influx of B cells into the skin-draining LN, which occurs after Day 7 and reduces the T:B ratio from 4:1 to 1:1 (S. L. Constant and R. A. Wilson, manuscript in

Table 1. T-cell-dependence of *in vitro* SSP-specific proliferation of LN cells from V20 mice

Lymph node	Days post-vaccination	Antibody *	C.p.m. in absence of antigen (+SEM)	D.c.p.m.	% inhibition of proliferation
Axillary	6	−	407 (±41)	35,495	
		+	165 (±15)	1869	95
Inguinal	6	−	492 (±23)	20,281	
		+	211 (±17)	3057	85
Mediastinal	20	−	770 (±76)	4829	
		+	1032 (±10)	10	100

* LN cells were treated with anti-Thy.1 antibody plus C (+), or C alone (−) before culture with APC and 100 μg/ml SSP.

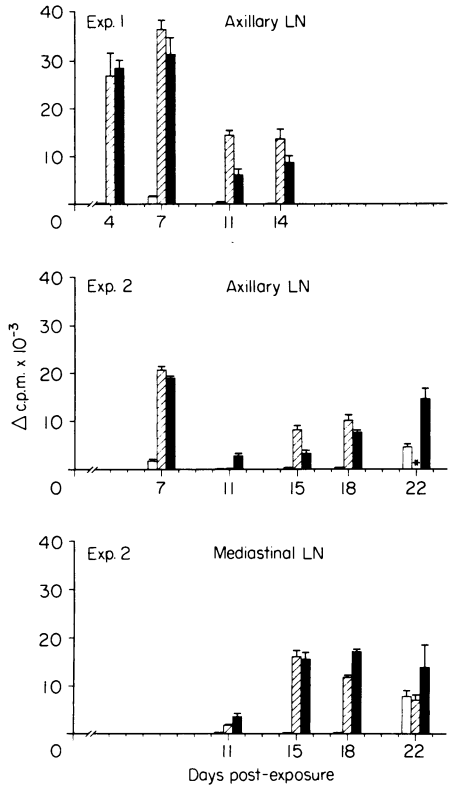

Figure 4. Proliferation of LN cells from naive (□), NI (▩) or V20 (■) mice cultured in the presence of 50 μg/ml SSP. *y*-axis, Δc.p.m. ± SEM for quadruplicate wells. * Not done.

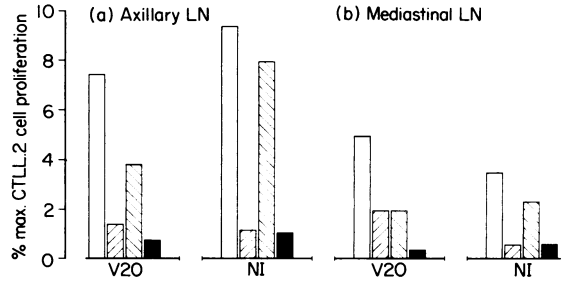

Figure 5. IL-2 and IL-4 production by (a) axillary LN cells 7 days or (b) mediastinal LN cells 22 days after exposure of NI or V20 mice to parasites. 72-hr supernatants from cells cultured with APC plus 50 μg/ml SSP were tested for their ability to induce proliferation of CTLL.2 cells in the presence of antibody to IL-2 (■), IL-4 (▧), both IL-2 and IL-4 (■) or in the absence of antibody (□).

preparation)[21], may also explain the decline in Th activity *in vitro*. T-cell reactivity in the mediastinal LN was detectable from around Day 10 onwards, coinciding with arrival of parasites in the lungs.[6] The comparatively low level of T-cell proliferative activity in the spleens of V20 mice appears to reflect the presence of very little parasite-released material, and the absence of parasites in this organ.[6]

The second wave of Th activity in skin-draining LN, leading to a peak at Day 18, may reflect antigen release by dead or dying parasites *in vivo*, or may be due to changes in levels of antigen-specific T cells in the LN. It is likely that T-cell responses measured in our *in vitro* assays reflect the kinetics of SSP release *in vivo*. However, there is no evidence, from ^{75}Se labelling of parasite proteins,[6] for a cyclical release of antigen, and similar kinetics were obtained when Lewis & Wilson[22] measured LN cell proliferation *in vitro* to antigen prepared from cercariae, adult worms or eggs.

Initial *in vitro* production of IFN-γ, predominantly by $CD4^+$ cells, from skin-draining and mediastinal LN coincided with T-cell proliferation and TCGF production. There was a general trend for higher IFN-γ production at later times (between Days 18 and 23), when proliferative and TCGF responses were declining. This was particularly marked in the spleen where the last two responses remained low at all times. Studies with T-cell lines and clones have shown that IFN-γ production does not require accompanying proliferation.[23,24] This suggests that, by Day 23, schistosome-specific T cells in the LN and those in the spleen will respond to antigen by releasing IFN-γ, but differ from recently stimulated cells in no longer proliferating upon contact with antigen.

Significantly higher levels of IFN-γ were produced in LN cell cultures of V20 than NI mice (up to ninefold in axillary LN, at Day 22). Since, after Day 7, there are approximately 24% fewer T cells in the axillary LN of V20 than of NI mice,[21] then equivalent numbers of V20 T cells should secrete 12-fold more IFN-γ than those in NI cultures. Additionally, the total number of cells in the axillary LN was consistently greater in V20 than in

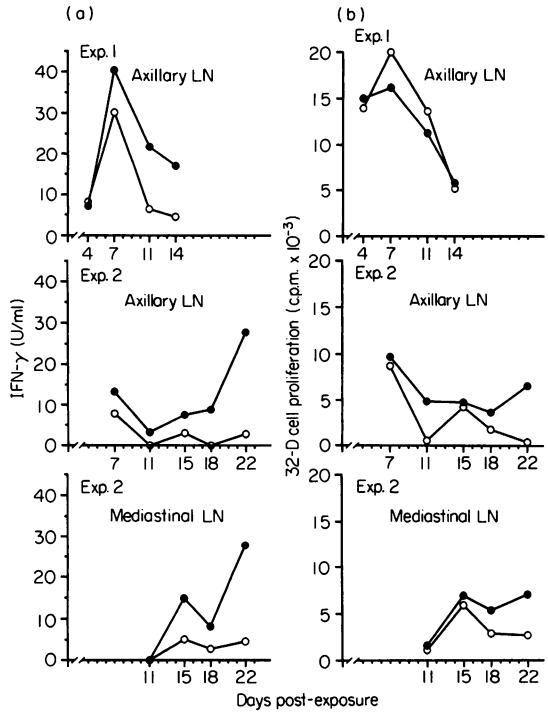

Figure 6. Measurement of IFN-γ (a) and IL-3 (b) in supernatants from axillary or mediastinal LN cells from NI (○) or V20 (●) mice after culture with APC plus 50 μg/ml SSP for 72 hr. IFN-γ release in the absence of SSP or from naive cells: Exp. 1, less than 9 U/ml; Exp. 2, less than 6·7 U/ml. ELISA sensitivity: Exp. 1, 8·5 U/ml; Exp 2, 5·3 U/ml. 32-D cell proliferation: supernatants from responder cells cultured without SSP or from naive cells gave less than 1000 c.p.m.

NI mice from Day 7 onwards (data not shown). Based on the observations of Constant *et al.*,[21] this represents twice as many T cells in the vaccinated group. The product of the above values gives an estimated 24-fold increase in total IFN-γ production from the axillary LN of V20 compared with NI mice at Day 22. Pearce *et al.*[25] have recently demonstrated that, after 3 weeks, more IFN-γ is produced by spleen cells from mice exposed to irradiated than to normal parasites.

Murine CD4+ Th cell clones have been divided into Th1 and Th2 subtypes, based on the lymphokines they produce.[26] Th1 clones secrete IL-2 and IFN-γ, whereas Th2 cells produce IL-4 and IL-5. In addition, freshly isolated Th0 clones have been identified,[16] which produce IL-2, IFN-γ and also IL-4. There has been speculation that a functional dichotomy occurs in Th responses to *S. mansoni*,[3] with protective vaccination inducing IFN-γ production by Th1 cells, whereas, in mice with patent bisexual infections, IL-5 production by Th2 cells predominates.[25] Resistance to challenge is partially depleted in mice treated with antibody to IFN-γ, but is unaffected in mice receiving antibody to IL-5.[27] Our results clearly support the notion that IFN-γ-producing CD4+ T cells play an important role in protective immunity. However, our data do not support the suggestion that responses induced by prepatent infection are protective in nature.[25] Indeed, we have observed a many-times greater capacity of T cells from LN of V20 mice to produce IFN-γ compared with NI mice. Furthermore, our observation that IL-4 production by cells from V20 mice is also elevated in comparison with NI mice indicates that Th2 responses are generated by vaccination (to date, we have no information on IL-5 production). In the absence of studies at the single cell level we are unable to determine whether IFN-γ and IL-4 are the products of distinct Th1 and Th2 cell subsets or if both cytokines are released from the same Th cells.[16,28]

The relevance, to protection, of large numbers of IFN-γ-producing T cells in the LN of V20 mice relies upon an extrapolation to events in the lungs. Here, challenge parasites generate inflammatory responses which are thought to block their migration.[29] Macrophages in the lungs are in a highly activated state following vaccination,[30] the likely result of IFN-γ production by the greater and sustained pulmonary infiltrate in V20 compared with NI mice.[31,32] The rapidity and magnitude of IFN-γ production by T cells in the lung following challenge infection (L. E. Smythies *et al.*, manuscript in preparation) suggests that CD4+ cells that take up residence and persist in the lungs after vaccination are memory cells.

It has been demonstrated[33,34] that antigen-specific memory T cells (expressing the Pgp-1 marker) produce more IFN-γ and IL-3 than naive cells upon antigenic stimulation, whereas IL-2 production remains constant. After Day 14, we have observed greater IL-3 production by axillary LN cells from V20 than NI mice and have reason to believe that these immune T cells develop a memory phenotype, since T cells from the lungs of vaccinated mice release IL-3 as well as IFN-γ, but not IL-2 (L. E. Smythies and R. M. Pemberton, unpublished observations). The enhanced generation of IFN-γ-producing cells in V20 over NI mice therefore appears to be integral to the development of memory T cells rather than populations of Th1 or Th2 subsets. This has yet to be confirmed by staining for the Pgp-1 marker.

The enhanced capacity of irradiated over normal parasites to elicit IFN-γ-producing T cells appears to reflect the ability of the former to act as their own adjuvant by persisting in the LN. A corollary would be that in mouse strains other than C57BL/6, which exhibit lower levels of resistance, less IFN-γ would be produced. The available evidence suggests that such a difference would not be accounted for by alterations in the kinetics of parasite migration.[6,35] It seems more likely that differences in the genetics of antigen presentation may lead to reduced IFN-γ production, or to increased Th2-like responses, acting antagonistically,[25] in less resistant mouse strains.

ACKNOWLEDGMENTS

We gratefully acknowledge the generosity of the following: DNAX corporation, Palo Alto, CA and Dr A. O'Garra, DNAX, for granting permission to use the S4B6 cell line; Dr G. Klaus and Mrs M. Holman, NIMR, Mill Hill, London, for kindly providing S4B6 and 11B11 cell lines; Dr E. A. Havell (The Trudeau Institute Inc., Saranac Lake, NY) for permission to use the R4-6A2 hybridoma; Dr A. Morris (Warwick University, U.K.) who provided the R4-6A2 hybridoma and the CHO 211A transfected cells, and Drs F. Y. Liew and J. Tite (The Wellcome Foundation, Beckenham, Kent, U.K.), who provided the R498 polyclonal antibody. Parasites were irradiated by Mrs P. Coulson, and Miss M. Howell provided technical assistance with cell culture. This work was funded by the E.E.C. Research Programme on Science and Technology for Development and the U.N.D.P./World Bank/W.H.O. Special Programme for Research and Training in Tropical Diseases.

REFERENCES

1. DEAN D.A. (1983) A review. *Schistosoma* and related genera: acquired resistance in mice. *Exp. Parasitol.* **55,** 1.
2. SCOTT P., PEARCE E., CHEEVER A.W., COFFMAN R.L. & SHER A. (1989) Role of cytokines and CD4+ T cell subsets in the regulation of parasite immunity and disease. *Immunol. Rev.* **112,** 161.
3. JAMES S.L. & SHER A. (1990) Cell-mediated immune responses to schistosomiasis. *Curr. Top. Microbiol. Immunol.* **155,** 21.
4. WILSON R.A. (1990) Leaky liver, portal shunting and immunity to schistosomes. *Parasitol. Today,* **6,** 354.
5. MANGOLD B.L. & DEAN D.A. (1984) The migration and survival of gamma-irradiated *Schistosoma mansoni* larvae and the duration of host-parasite contact in relation to the induction of resistance in mice. *Parasitology,* **88,** 249.
6. MOUNTFORD A.P., COULSON P.S. & WILSON R.A. (1988) Antigen localization and the induction of resistance in mice vaccinated with irradiated cercariae of *Schistosoma mansoni. Parasitology,* **97,** 11.
7. MOUNTFORD A.P. & WILSON R.A. (1990) *Schistosoma mansoni*: the effect of regional lymphadenectomy on the level of protection induced in mice by radiation-attenuated cercariae. *Exp. Parasitol.* **71,** 463.
8. KELLY E.A.B. & COLLEY D.G. (1988) *In vivo* effects of monoclonal anti-L3T4 antibody on immune responsiveness of mice infected with *S. mansoni.* Reduction of irradiated cercariae-induced resistance. *J. Immunol.* **140,** 2737.
9. VIGNALI D.A.A., CROCKER P., BICKLE Q.D., COBBOLD S., WALDMANN H. & TAYLOR M.G. (1989) A role for CD4+ but not CD8+ T cells in immunity to *Schistosoma mansoni* induced by 20k rad-irradiated and Ro 11-3128-terminated infections. *Immunology,* **67,** 466.
10. JAMES S.L., CORREA-OLIVEIRA R. & LEONARD E.J. (1984) Defective vaccine-induced immunity to *Schistosoma mansoni* in P strain mice. II. Analysis of cellular responses. *J. Immunol.* **133,** 1587.
11. RAMALHO-PINTO F.J., GAZZINELLI G., HOWELLS R.E., MOTA-SANTOS T.A., FIGUEIREDO E.A. & PELLEGRINO J. (1974) *Schistosoma mansoni*: defined system for stepwise transformation of cercaria to schistosomule *in-vitro. Exp. Parasitol.* **36,** 360.
12. LAZDINS J.K., STEIN M.J., DAVID J.R. & SHER A. (1982) *Schistosoma mansoni*: Rapid isolation and purification of schistosomula of different developmental stages by centrifugation on discontinuous density gradients of Percoll. *Exp. Parasitol.* **53,** 39.
13. ZURAWSKI S.M., MOSMANN T.R., BENEDIK M. & ZURAWSKI G. (1986) Alterations in the amino-terminal third of mouse interleukin-2: effects on biological activity and immunoreactivity. *J. Immunol.* **137,** 3354.
14. OHARA J. & PAUL W.E. (1985) Production of a monoclonal antibody to and molecular characterization of B cell-stimulatory-factor-1. *Nature (Lond.),* **315,** 333.
15. GILLIS S. & SMITH K.A. (1977) Long term culture of tumor-specific cytotoxic T cells dependent on IL-2. *Nature (Lond.),* **268,** 154.
16. FIRESTEIN G.S., ROEDER W.D., LAXER J.A., TOWNSEND K.S., WEAVER C.J., HOM J.T., LINTON J., TORBETT B.E. & GLASEBROOK A.L. (1989) A new murine CD4+ T cell subset with an unrestricted cytokine profile. *J. Immunol.* **143,** 518.
17. HAMBLIN A.S. & O'GARRA A. (1987) Assays for interleukins and other related factors. In: *Lymphocytes, A Practical Approach* (ed. G.G.B. Klaus), Chap. 10, p. 209. IRL Press, Oxford.
18. SPITALNY G.L. & HAVELL E.A. (1984) Monoclonal antibody to murine gamma interferon inhibits lymphokine-induced antiviral and macrophage tumouricidal activities. *J. exp. Med.* **159,** 1560.
19. HUTCHINGS P.R., CAMBRIDGE G., TITE J., MAEGER A. & COOKE A. (1989) The detection and enumeration of cytokine-secreting cells in mice and man and the clinical application of these assays. *J. immunol. Meth.* **120,** 1.
20. MORRIS A.G. & WARD G. (1987) Production of recombinant interferons by expression in heterologous mammalian cells. In: *Interferons and Lymphokines, a Practical Approach* (eds M. J. Clemens, A. G. Morris & A. J. H. Gearing), Chap. 5, p. 61. IRL Press, Oxford.
21. CONSTANT S.L., MOUNTFORD A.P. & WILSON R.A. (1990) Phenotypic analysis of the cellular responses in regional lymphoid organs of mice vaccinated against *Schistosoma mansoni. Parasitology,* **101,** 15.
22. LEWIS F.A. & WILSON E.M. (1982) Regional and splenic lymphocyte proliferative responses of mice exposed to normal or irradiated *S. mansoni* cercariae. *Am. J. Trop. Med. Hyg.* **31,** 505.
23. KELSO A., GLASEBROOK A.L., KANAGAWA O. & BRUNNER K.T. (1982) Production of macrophage-activating factor by T lymphocyte clones and correlation with other lymphokine activities. *J. Immunol.* **129,** 550.
24. HECHT T.T., LONGO D.L. & MATIS D.L. (1983) The relationship between immune interferon production and proliferation in antigen-specific MHC-restricted T-cell lines and clones. *J. Immunol.* **131,** 1049.
25. PEARCE E.J., CASPAR P., GRZYCH J.-M., LEWIS F.A. & SHER A. (1991) Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, *Schistosoma mansoni. J. exp. Med.* **173,** 159.
26. MOSMANN T.R. & COFFMAN R.L. (1987) Two types of mouse helper T cell clones. Implications for immune regulation. *Immunol. Today,* **8,** 223.
27. SHER A., COFFMAN R.L., HIENY S. & CHEEVER A.W. (1990) Ablation of eosinophil and IgE responses with anti-IL-5 or anti-IL-4 antibodies fails to affect immunity against *Schistosoma mansoni* in the mouse. *J. Immunol.* **145,** 3911.
28. KELSO A. & GOUGH N.M. (1988) Coexpression of granulocyte-macrophage colony-stimulating factor, g-IFN, and interleukins 3 and 4 is random in murine alloreactive T-lymphocyte clones. *Proc. natl. Acad. Sci. U.S.A.* **85,** 9189.
29. WILSON R.A. & COULSON P.S. (1989) Lung phase immunity to schistosomes: a new perspective on an old problem. *Parasitol. Today,* **5,** 274.
30. MENSON E.N. & WILSON R.A. (1989) Lung phase immunity to *Schistosoma mansoni.* Flow cytometric analysis of macrophage activation states in vaccinated mice. *J. Immunol.* **143,** 2342.
31. AITKEN R., COULSON P.S. & WILSON R.A. (1988) Pulmonary leukocyte responses are linked to the acquired immunity of mice vaccinated with irradiated cercariae of *Schistosoma mansoni. J. Immunol.* **140,** 3573.
32. SMYTHIES L.E. & WILSON R.A. (1991) Interferon-gamma, pulmonary inflammation and immunity to schistosomes. British Cytokine Group Meeting, 9 Nov 1990. *Int. J. Exp. Pathol.* **72,** A1.
33. BUDD R.C., CEROTTINI J.-C. & MACDONALD H.R. (1987) Selectively increased production of interferon-γ by subsets of Lyt.2+ and L3T4+T cells identified by expression of PgP-1. *J. Immunol.* **138,** 3583.
34. SANDERS M.E., MAKGOBA M.W. & SHAW S. (1988) Human and murine memory T cells: reinterpretation of helper-inducer and suppressor-inducer subsets. *Immunol. Today,* **9,** 195.
35. MASTIN A.J., BICKLE Q.D. & WILSON R.A. (1983) *Schistosoma mansoni*: Migration and attrition of irradiated and challenge schistosomula in the mouse. *Parasitology,* **87,** 87.