INFECTION AND IMMUNITY, July 1996, p. 2556–2562 Vol. 64, No. 7
0019-9567/96/$04.00+0

Immunity to *Cryptosporidium muris* Infection in Mice Is Expressed through Gut CD4$^+$ Intraepithelial Lymphocytes

VINCENT McDONALD,* HEIDI A. ROBINSON, JOHN P. KELLY, AND GREGORY J. BANCROFT

Department of Clinical Sciences, London School of Hygiene and Tropical Medicine, London WC1E 7HT, United Kingdom

Received 31 January 1996/Returned for modification 19 March 1996/Accepted 3 April 1996

The role of gut intraepithelial lymphocytes (IEL) in immunity to cryptosporidial infection was investigated with a murine infection model involving *Cryptosporidium muris*. Oocyst shedding was monitored in severe combined immunodeficiency (SCID) mice infected with *C. muris* following intravenous injection of mesenteric lymph node (MLN) cells or intestinal IEL from BALB/c donor mice which were naive or previously infected with *C. muris*. SCID mice receiving no lymphoid cells developed chronic infections and excreted large numbers of oocysts until the end of the experiment. SCID mice injected with IEL from immune animals, however, were able to overcome the infection, and furthermore, these animals produced fewer oocysts and recovered sooner than ones which received IEL or MLN cells from naive BALB/c donors. Similar levels of protection were obtained in SCID mice injected with either 2×10^6 IEL or MLN cells from immune donor mice. Depletion of CD4$^+$ cells from immune IEL, however, abrogated the ability to transfer immunity to SCID mice, while depletion of CD8$^+$ cells only marginally reduced the protective capacity of immune IEL. Finally, control SCID mice which received no lymphocytes had ≤1% CD4$^+$ cells in the IEL from the small intestine, whereas the IEL from SCID mice recovered from infection, as a result of injection with immune IEL, contained 15% CD4$^+$ cells. Thus, the ability to control *C. muris* infection correlated with the presence of the protective CD4$^+$ cells in the gut epithelium.

Cryptosporidiosis is an important mucosal disease of vertebrates caused by coccidial parasites of the genus *Cryptosporidium*. The parasite develops in epithelial cells, and infection is transmitted by oocysts, normally in a fecal-oral manner. Infection of mammals with *Cryptosporidium parvum* produces a watery diarrhea which normally lasts several days, but immunodeficient hosts, including individuals with AIDS, often have chronic and life-threatening symptoms (5). Another species of parasite, *Cryptosporidium muris*, infects the gastric glands of rats and mice and is less pathogenic than *C. parvum* (13, 25). In rodents, recovery from cryptosporidial infection requires T-cell activity (12, 16, 19, 26), particularly from the CD4$^+$ helper cell subpopulation (4, 15, 17, 20, 26, 27). Although the CD8$^+$ cytotoxic/suppressor cells are not necessary for control of infection (15, 20), we have nevertheless observed a minor role for these cells in immunity (15, 17). The effector mechanisms mediating elimination of *Cryptosporidium* infection are not understood, although it is clear that gamma interferon (IFN-γ) plays an important part (4, 16, 27).

Little information on the measurement of protective immune responses against *Cryptosporidium* spp. at the mucosal level is available. The lymphoid cells of the gut-associated lymphoid tissue likely to be responsible for the control of the parasite's reproduction are found in three distinct compartments: the connective tissue of the lamina propria, specialized lymphoid tissue such as Peyer's patches, and the epithelium (18). The lymphocytes from each compartment differ in ontogeny as well as in stages of activation and maturation. Intraepithelial lymphocytes (IEL) might be expected to form a first line of defense against pathogens affecting the mucosal surface, such as cryptosporidia. Indeed, increased numbers of intestinal IEL have been observed during coccidial infections of the gastrointestinal tract, including *C. parvum* infections (3, 9, 10), but the importance of these cells in the protective immune response has not been determined. The composition of lymphocyte subsets found in gastrointestinal IEL of the mouse has been reported to vary with the strain and age of the mice, the region of the gut, and even housing conditions (1). In the small intestine, IEL have been found to be predominantly CD3$^+$ T cells, with CD8$^+$ cells more prevalent than CD4$^+$ cells; in addition, T cells with T-cell receptor γδ (TCR-γδ) are more common than in other lymphoid compartments, and there are few or no B cells (22). The function of IEL in the control of mucosal infections is unclear at present, but in vitro, these cells have demonstrated effector mechanisms characteristic of T cells and upon appropriate stimulation released T-cell-derived cytokines (22, 23).

The present investigation was designed to determine whether IEL were capable of eradicating cryptosporidial infection. We had shown previously that immunity to *C. muris* infection could be adoptively transferred from previously infected BALB/c mice to severe combined immunodeficiency (SCID) mice which lack T and B cells (2), using either mesenteric lymph node (MLN) or spleen cells (17). In the present study, this approach of adoptive immunization was employed to characterize the protective nature of IEL from immunocompetent animals used as donor cells. The results show that IEL obtained from immune donor mice can adoptively transfer immunity against *C. muris* and that the protection observed is associated predominantly with the CD4$^+$ cell subpopulation.

MATERIALS AND METHODS

Animals. CB.17 mice homozygous for the *scid* mutation (2) were bred under aseptic conditions at the London School of Hygiene and Tropical Medicine and used at 12 weeks of age. In experiments involving adoptive transfer of immunity, SCID mice of the same sex and between 8 and 12 weeks old were maintained in clean rooms. The mice, used in groups of six for each treatment, were housed individually in wire-floored cages with filter lids and supplied with food and water

* Corresponding author. Mailing address: London School of Hygiene & Tropical Medicine, Department of Clinical Sciences, Keppel St., London WC1E 7HT, United Kingdom. Phone: 0171 927 2291. Fax: 0171 637 4314.

sterilized by radiation and autoclaving, respectively. BALB/c female mice of similar ages were obtained from Charles River U.K. Ltd.

Parasite. The RN 66 strain of *C. muris* (13) was passaged in SCID mice, and oocysts were purified by salt flotation and stored at 4°C in a 2.5% potassium dichromate solution (16). Experimental infections were initiated by oral inoculation of mice with 2×10^5 oocysts, and production of oocyst progeny in feces was measured daily after collection of pellets as described previously (16). Pellets were homogenized in 20 ml of water, and samples were examined microscopically in a hemocytometer after a further dilution of 1:3 in water.

Preparation of lymphoid cell suspensions. Cell suspensions of MLN cells from BALB/c mice previously infected or not infected (naive controls) with *C. muris* were prepared in RPMI 1640 medium (GIBCO) with 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), 200 U of penicillin per ml, 200 μg of streptomycin per ml, and 10% fetal calf serum (FCS) (GIBCO). IEL were prepared from small intestines by a procedure based on those previously described by other workers (1, 6). In summary, the small intestines were cleaned of fecal debris by being flushed with 10 ml of ice-cold Ca^{2+}- and Mg^{2+}-free Hanks' solution (CMFH) (GIBCO) with 2% FCS. Gut segments of approximately 1 cm^2 from four or five mice were pooled in 50-ml flasks (Falcon) containing 25 ml of CMFH with 10% FCS and 0.1 mM EDTA warmed to 37°C. Following a 20-min incubation at 37°C and vigorous shaking of the flask by hand for 25 s, the medium was removed. This part of the procedure was repeated to release any remaining epithelium into the medium. The medium containing IEL and epithelial cell debris was centrifuged at 1,000 rpm (Centaur2), and the cells were resuspended in 5 ml of cold RPMI 1640 medium with antibiotics (see above) before being passed through a nylon wool column. The column was immediately washed through with 5 ml of cold RPMI 1640 medium, and the collected cell suspension was centrifuged and resuspended in 40% Percoll (Sigma). The cells in 40% Percoll were layered over 70% Percoll in a 15-ml conical tube and centrifuged at 2,700 rpm for 20 min. The band of cells containing IEL was collected from the interface between 40 and 70% Percoll and washed three times in 10% FCS–RPMI 1640 medium. The viability was measured by trypan blue exclusion, and the purified cells were found to be greater than 90% viable. Prior to infection, mice were injected intravenously with 2×10^6 viable cells, except in the experiment to titrate the protective effect of IEL (see Fig. 2).

Depletion of T-cell subsets. $CD4^+$ and $CD8^+$ cells were depleted from IEL by treatment with rat monoclonal antibodies (MAbs) (SeraLab) specific for the relevant surface markers by using protocols recommended by the manufacturers. The cells were adjusted to a concentration of 10^7/ml and incubated at room temperature for 15 min with a 1:50 dilution of YTS 191.1 (anti-L3/T4), YTS 169.4 (anti-Lyt-2), or a nonreactive rat immunoglobulin G (IgG). The cells, with antibodies, were then injected intravenously into mice.

Immunofluorescence staining and cytometric analysis. Cell suspensions containing 10^6 cells were added to plastic tubes (12 by 75 mm), washed in phosphate-buffered saline (PBS) with 2% FCS and 0.01% sodium azide, and resuspended in 100 μl of normal rabbit serum for 10 min at 4°C to prevent nonspecific staining by antibodies added later. The cells were then washed once and incubated for 30 min at 4°C in 100 μl of a rat MAb (1:100 dilution) against the mouse lymphocyte surface marker CD4 (phycoerythrin conjugated), CD8 (fluorescein isothiocyanate conjugated), or Ig (fluorescein isothiocyanate conjugated) (all from Sigma). For detection of TCR-αβ, cells first were incubated with biotin-conjugated hamster anti-mouse TCR-αβ IgG or an isotype-matched control antibody (1:100 dilution; PharMingen) and then, following washing, were incubated for 30 min with avidin-conjugated quantum red. Following a further two washes, all cell preparations labelled with antibodies were fixed by incubation at room temperature with 2% paraformaldehyde in PBS and then washed twice in PBS and analyzed by FACScan (Becton Dickinson). The cells were gated for lymphocytes, which represented more than 90% of the IEL population.

Histology. Fresh tissues were fixed in formal saline and embedded in paraffin wax, and 5-μm-thick sections were prepared and stained with hematoxylin and eosin.

Statistics. Standard errors are shown on the figures, and data were analyzed with Student's *t* test.

RESULTS

Infection of immunocompetent mice. BALB/c mice used as donors in adoptive transfer of immunity were immunized against *C. muris* by infection following inoculation with 2×10^5 oocysts. Oocyst shedding was first detectable around day 10 postinfection (p.i.), with the level increasing until between days 14 and 18 p.i. and then declining until infections became subpatent by day 25. It was previously found that animals recovered from infection demonstrated no patent infection more than 30 days following challenge with 10^6 oocysts of the homologous parasite strain (16). In experiments involving adoptive transfer of immunity, MLN cells and IEL were obtained from infection-immunized mice between days 63 and 88 p.i. for transfer to SCID mice.

Analysis of IEL from immune and naive BALB/c mice. Studies were done to determine whether the IEL isolated were likely to be contaminated with lymphocytes from the lamina propria and also to compare the lymphocyte subpopulation compositions of IEL from immune and naive donor mice. Intestinal segments were examined histologically after the procedure to remove the epithelium had been performed. Few epithelial cells remained attached to the villi, but the villous architecture and basal membrane appeared to have remained intact (results not presented). Flow cytometry was used to determine the prevalence of major lymphocyte subpopulations present in isolated IEL. The typical murine IEL from the small intestine have a predominance of $CD8^+$ cells over $CD4^+$ cells and have few Ig^+ cells (1, 22). In the present study, the IEL from naive BALB/c mice were 92% $CD8^+$ cells, 25% $CD4^+$ cells, 81% TCR-$\alpha\beta^+$ cells, and ≤1% Ig^+ cells. These results indicated that IEL contained little or no contamination with cells from the lamina propria. Similar corresponding values were obtained when the same lymphocyte subsets were observed in IEL from BALB/c mice infected with *C. muris* 64 days previously.

Adoptive transfer of immunity. *C. muris* infections in groups of SCID mice following injection of IEL or MLN cells from naive or immune BALB/c mice were compared (Fig. 1). Oocyst shedding in all groups of mice became patent on day 11 p.i. and increased sharply over the next few days, reaching a mean peak level in most groups on day 17. SCID mice which received no lymphoid cells developed chronic infections and continued to shed large numbers of oocysts throughout the remainder of the experiment. In contrast, mice which received IEL from immune donors recovered from infection; furthermore, these mice had lower peak values of oocyst excretion and recovered sooner than mice given naive lymphoid cells. MLN cells from immune donors provided a level of protection comparable to that obtained with IEL from the same animals. Subpatent infections were eventually obtained in all mice given naive MLN cells, but infections remained patent in mice given naive IEL up to at least day 54 p.i. In another, similar experiment, three of five SCID mouse recipients of naive IEL still had heavy patent infections 140 days p.i. (data not presented). The total mean numbers of oocysts produced until day 54 p.i. by SCID mice receiving immune IEL ($11.2 \times 10^6 \pm 2.2 \times 10^6$) or immune MLN cells ($9.2 \times 10^6 \pm 5.8 \times 10^6$) were significantly less than the values obtained for mice receiving naive IEL ($134.6 \times 10^6 \pm 35.0 \times 10^6$) or MLN cells ($65.0 \times 10^6 \pm 28.8 \times 10^6$), while the greatest production was found in the SCID mouse control group which received no donor lymphocytes ($211.4 \times 10^6 \pm 28.0 \times 10^6$) ($P < 0.001$). Although there was little difference between the levels of oocyst shedding by groups given immune IEL and immune MLN cells, there was a significant difference between the yields from groups given naive IEL and naive MLN cells ($P < 0.001$). These results demonstrated that IEL could be used to transfer immunity against *C. muris* adoptively from immune BALB/c mice to histocompatible SCID mice.

Titration of immune IEL. Compared with MLN or spleen cells, IEL are more difficult to harvest, and significantly fewer cells may be isolated. In our experience, 2×10^6 to 5×10^6 IEL were obtained from the small intestine of a BALB/c mouse, and previous infection did not affect the numbers that could be isolated. It was necessary, therefore, to determine a minimal number of infection-primed IEL which could transfer immunity to SCID mice. Accordingly, prior to infection with *C. muris*, groups of SCID mice received either 10^4, 10^5, or 10^6

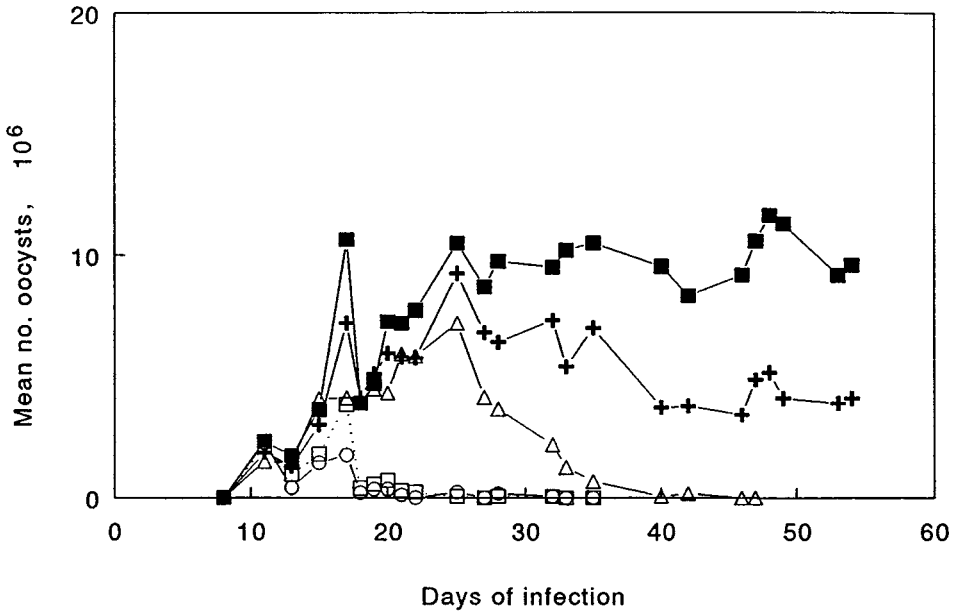

FIG. 1. Adoptive transfer of immunity to *C. muris* in SCID mice reconstituted with MLN cells or IEL from previously infected BALB/c mice. Oocyst production was measured in groups of mice injected intravenously with infection-primed or naive immunocompetent cells prior to infection. The groups were SCID controls (■), naive IEL recipients (+), naive MLN cell recipients (△), immune MLN cell recipients (○), and immune IEL recipients (□).

immune IEL, while control groups received either 10^6 naive IEL or no lymphoid cells. The transfer of immunity was observed only in the group which received 10^6 immune IEL (Fig. 2). These animals began recovery from infection after day 21 p.i., while high levels of chronic infection were observed in all other groups throughout the remainder of the monitoring period.

Role of $CD4^+$ and $CD8^+$ cells in transfer of immunity with IEL. Experiments to measure the involvement of the $CD4^+$ and $CD8^+$ T-cell subsets of IEL in adoptive immunity. In the first experiment, a comparison of the protective effects of immune IEL depleted of $CD4^+$ cells, the unfractionated immune cell population, and naive IEL was made (Fig. 3). As before, recipients of naive IEL developed chronic infections, producing a mean of $78.3 \times 10^6 \pm 30.8 \times 10^6$ oocysts up to day 50 p.i., while animals receiving immune IEL had subpatent infections by day 30 and produced only $10.7 \times 10^6 \pm 1.8 \times 10^6$ oocysts during the patent period. However, when the immune IEL had been depleted of $CD4^+$ cells, none of the recipients showed evidence of control of infection, producing $106.1 \pm 13.5 \times 10^6$

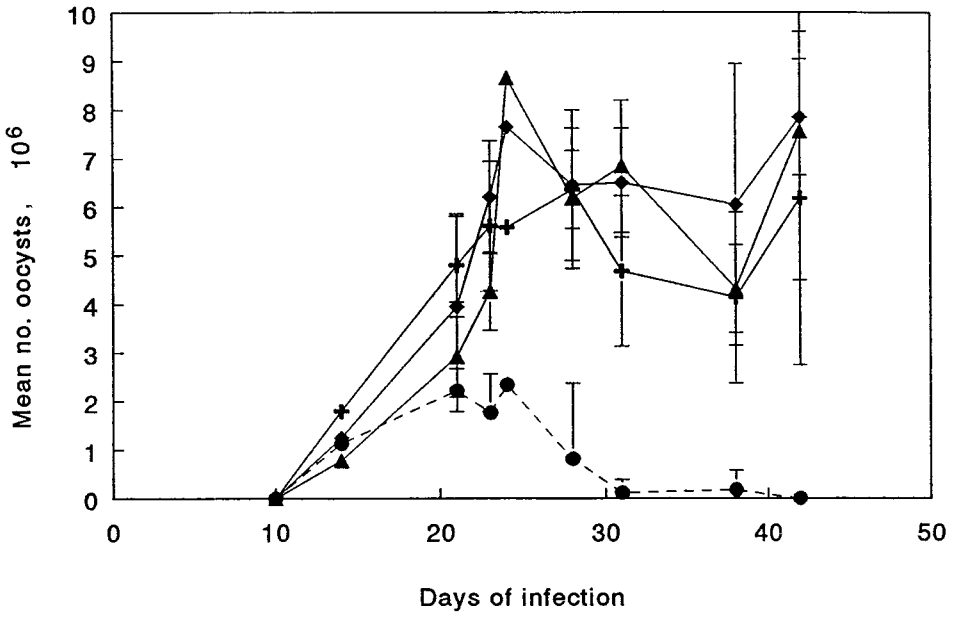

FIG. 2. Titration of protective effect of immune IEL cells against *C. muris* in SCID mice. The mice received either 10^6 naive IEL (+), 10^6 immune IEL (●), 10^5 immune IEL (▲), or 10^4 immune IEL (◆).

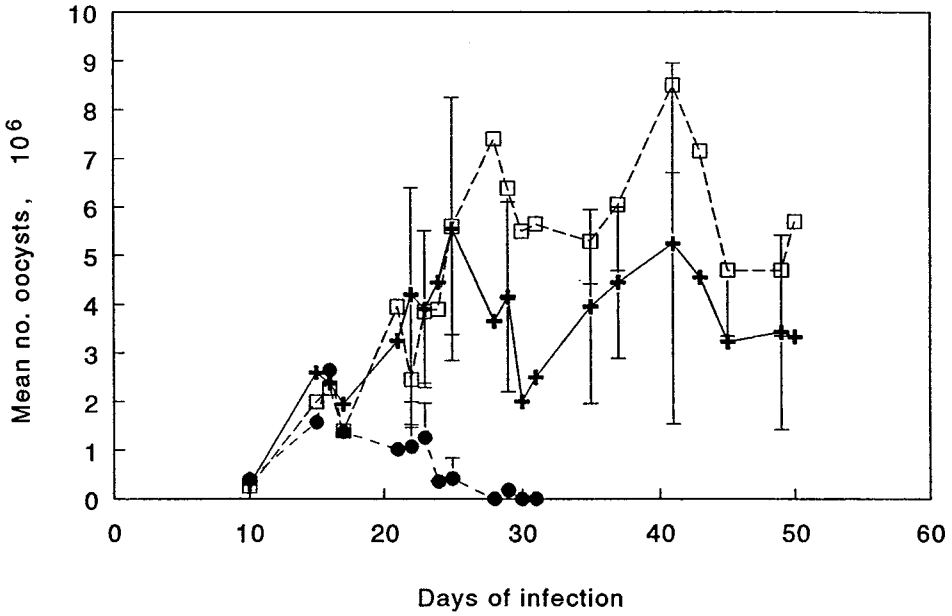

FIG. 3. Effect of depletion of $CD4^+$ cells from immune IEL on the adoptive transfer of immunity to *C. muris*. SCID mice received 2×10^6 naive IEL (+), immune IEL (●), or immune IEL depleted of $CD4^+$ cells following incubation with a rat anti-mouse CD4 MAb (□).

oocysts by day 50; these animals were as susceptible to infection as the recipients of naive IEL.

In another experiment, a comparison of the effects of depleting $CD4^+$ cells and $CD8^+$ cells from immune donor cells was made (Fig. 4). SCID mice given immune IEL depleted of $CD4^+$ cells again failed to control oocyst production, whereas those receiving immune IEL depleted of $CD8^+$ cells were able to recover from infection. Patent infections in mice given immune IEL depleted of $CD8^+$ cells lasted longer than those in the recipients of unfractionated immune IEL (29.6 ± 4.4 days compared with 22.0 ± 7.9 days; $P < 0.05$); in addition, the total mean yield of oocysts from the recipients of immune IEL depleted of $CD8^+$ cells ($41.7 \times 10^6 \pm 24.6 \times 10^6$) was also greater than that from the recipients of unfractionated immune IEL ($16.4 \times 10^6 \pm 10.4 \times 10^6$), but the difference was not statistically significant. These results demonstrated that $CD4^+$ cells were predominantly responsible for the protection against *C. muris* infection conferred by immune IEL, while $CD8^+$ cells were of measurable but minor importance.

Homing of donor IEL to the gut in SCID mice. To determine

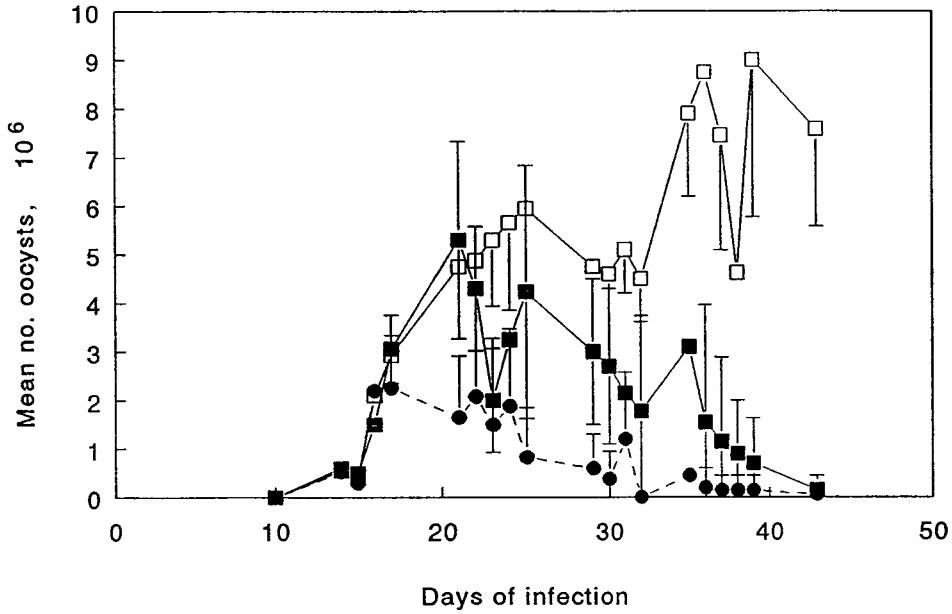

FIG. 4. Adoptive transfer of immunity against *C. muris* and effect of depleting $CD4^+$ or $CD8^+$ cells from immune donor IEL. The T-cell subpopulations were depleted following incubation with specific rat MAbs. Groups of SCID mice received 2×10^6 immune IEL which were either unfractionated (●), depleted of $CD4^+$ cells (□), or depleted of $CD8^+$ cells (■).

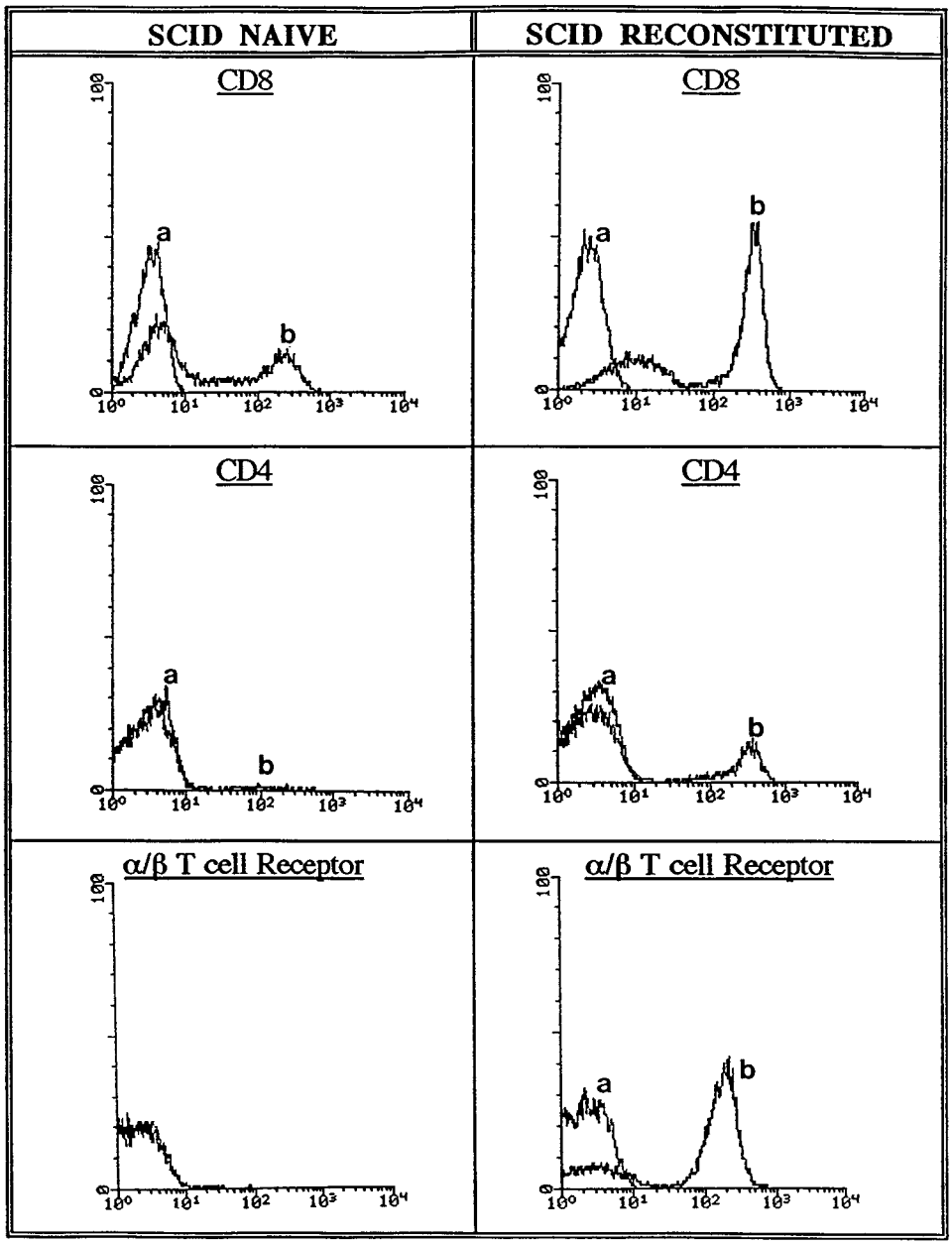

FIG. 5. Flow cytometry analysis of T-cell subpopulations present in IEL from naive SCID mice and SCID mice reconstituted with immune IEL. The IEL from reconstituted mice were obtained from the mice for which results are shown in Fig. 4 following recovery from infection. The cells were incubated with a control antibody (a) or with an antibody to detect CD8, CD4, or TCR-αβ (b).

whether immune IEL were able to populate the epithelia of recipient SCID mice, an examination was made of the lymphocyte composition of IEL from SCID mice previously reconstituted with immune IEL recipients (those for which results are shown in Fig. 4), 14 days after clearance of the patent infection (Fig. 5). These IEL were 68% $CD8^+$ cells, 77% TCR-$\alpha\beta^+$ cells, ≤1% Ig^+ cells, and 15% $CD4^+$ cells. The IEL from naive (uninfected) SCID mice, however, contained ≤1% $CD4^+$ cells and TCR-$\alpha\beta^+$ cells, while 33% were $CD8^+$ cells, as would be expected (18). In separate experiments, SCID mice with chronic infections had $CD4^+$ and TCR-$\alpha\beta^+$ cell numbers which were not significantly above the background level (data not presented). Recovery from infection in SCID mice which received immune IEL therefore correlated with the presence of $CD4^+$ cells in the gastrointestinal epithelium.

DISCUSSION

In vitro studies have suggested that IEL may have an important regulatory and/or effector function in immunity to mucosal infection (18, 22), but confirmation for this from in vivo investigation has been lacking. Since *Cryptosporidium* development is confined to the epithelium, our *C. muris* model seemed suitable for the investigation of the involvement of IEL in immunity to an intracellular mucosal pathogen. Our demonstration that immunity against *C. muris* could be adoptively

transferred with IEL indicated that in the murine host these lymphocytes were, indeed, important effectors of immunity. These results are the first to show that IEL could be primed against cryptosporidial infection and also that the primed IEL could induce elimination of this parasite.

The lymphocytes isolated from the epithelium for adoptive transfer of immunity appeared to contain few or no lamina propria lymphocytes, as evidenced by the relative numbers of $CD4^+$, $CD8^+$, and Ig^+ cells present and also by the apparent intact nature of the villous architecture following removal of the epithelium. This suggested that the IEL and not contaminating lamina propria cells were predominantly responsible for adoptive immunization against the cryptosporidial parasite. The IEL donor cells were obtained from mice which had cleared a patent infection up to about 60 days previously, indicating that the immunological memory of these cells was long lived.

Within the IEL population, $CD4^+$ cells were required for the transfer of immunity, since depletion of these cells abrogated the protective effect following transfer. In comparison, depletion of $CD8^+$ cells resulted in only a small reduction in the level of immunity obtained with immune IEL, suggesting that this subset of cells had a limited role in control of infection. It is possible that the cells with the CD8 phenotype involved in protection may come from a double-positive $CD4^+$ $CD8^+$ cell population found in IEL which were found in an earlier study by other workers to repopulate the gut epithelium following transfer to SCID mice (21). The observations here on the relative importance of $CD4^+$ and $CD8^+$ cells from IEL in the adoptive transfer of immunity were consistent with our earlier results showing that in the control of *C. muris* infection, $CD4^+$ cells played a more important part than $CD8^+$ cells (17).

While immunity could be adoptively transferred with IEL from immune donors, IEL from naive BALB/c donor mice conferred a relatively poor level of protection on SCID mice. The relative numbers of $CD4^+$, $CD8^+$, TCR-$\alpha\beta^+$, and Ig^+ cells were found to be similar in immune and naive IEL, indicating that any difference between these populations in the ability to transfer immunity to SCID mice was unlikely to reflect a discrepancy in the corresponding numbers of these major cell subsets. In the present study, as in a previous one (17), SCID mice injected with naive MLN cells were able to control *C. muris* infection, although less effectively than immune MLN cells. It is not clear why in this experimental system naive MLN cells were more protective than IEL from the same donor animals. However, there is evidence to suggest that IEL are predominantly resting memory cells (18), and so the "naive" population transferred to SCID mice might be expected to be largely unresponsive to *C. muris.*

Recovery from infection by SCID mice injected with immune IEL correlated with the presence in the small intestinal epithelium of significant numbers of $CD4^+$ cells, which suggested that the impact of these cells on the infection might be dependent on a close interaction with the epithelium. This repopulation of the gut epithelium with $CD4^+$ cells was consistent with observations of other workers, who recently showed that BALB/c IEL transferred to SCID mice readily repopulated the intestinal epithelium (21). Preliminary attempts to isolate IEL from the stomach for phenotype analysis were unsuccessful (data not presented), and it may be necessary to employ immunohistological methods to evaluate homing of lymphocytes to the site of infection. Our finding that immune donor IEL obtained from the small intestine conferred immunity against an infection (apparently) confined to the gastric mucosa indicated that lymphocytes primed in the stomach were able to migrate through the common mucosal system to occupy distant sites in the gut (18).

The mechanisms whereby IEL induced control of *C. muris* development are unknown. IEL have a cytotoxic capacity (11, 18, 22), and intestinal infection with *Toxoplasma gondii* resulted in the generation of cytotoxic $CD8^+$ TCR-$\alpha\beta^+$ IEL which, in vitro, specifically lysed parasite-infected epithelial cells with major histocompatibility complex class I restriction (14). IEL contain higher numbers of $CD8^+$ TCR-$\gamma\delta^+$ cells than there are in other lymphocyte compartments, but their role in immunity is unclear (18, 22). However, $CD8^+$ IEL played only a small part in control of *C. muris* infection, so the effector mechanisms involving these cells are unlikely to be crucial.

Generally, in vitro functional studies of T cells from IEL have shown that they are less susceptible to activation than T cells from other sites. However, they were able to proliferate in the presence of T-cell mitogens, and stimulation resulted in the release of T-cell cytokines (22, 23). IEL from animals infected with the intestinal pathogens *Trichinella spiralis* and *Listeria monocytogenes* secreted a number of cytokines, including IFN-γ, in the presence of specific antigen (7, 28). We have shown that the onset of immunity to *C. muris* infection was significantly delayed and oocyst excretion was increased following treatment with anti-IFN-γ antibodies (16), and, in another study, the development of immunity correlated with production of IFN-γ and interleukin-2 by MLN cells and splenic $CD4^+$ cells (24). Other investigators have shown that IFN-γ played an important part in the $CD4^+$ cell-mediated elimination of *C. parvum* infection (4, 27). The mechanisms of action of IFN-γ are not known, but it has been shown to inhibit the in vitro development of *T. gondii* and *Eimeria tenella* in cell lines (8, 14).

In conclusion, the results presented here showed that $CD4^+$ IEL from immunocompetent mice previously infected with *C. muris* induced immune effector mechanisms responsible for the elimination of this parasite. Future studies of immunity to *C. muris* infection will include in vitro and in vivo investigations of the functional characteristics of immune IEL, particularly the protective $CD4^+$ cell subpopulation.

ACKNOWLEDGMENTS

This work was funded by the Medical Research Council of Great Britain.

We thank M. E. Rose, Institute of Animal Health, Compton, Berks, United Kingdom, for advice on the method for isolation of IEL.

REFERENCES

1. **Beagley, K. W., K. Fujihashi, A. S. Lagoo, S. Lagoo-Deenadaylan, C. A. Black, A. M. Murray, A. T. Sharmanov, M. Yamamoto, J. R. McGhee, C. O. Elson, and H. Kiyoni.** 1995. Differences in intraepithelial lymphocyte T cell subsets isolated from murine small versus large intestine. J. Immunol. **154:** 5611–5619.
2. **Bosma, G. C., R. P. Custer, and M. J. Bosma.** 1983. A severe combined immunodeficiency mutation in the mouse. Nature (London) **301:**527–530.
3. **Chardes, T., D. Buzoni-Gatel, A. Lepage, F. Bernard, and D. Bout.** 1994. *Toxoplasma gondii* oral infection induces specific cytotoxic $CD8\alpha/\beta^+$ Thy-1^+ gut intraepithelial lymphocytes, lytic for parasite-infected enterocytes. J. Immunol. **153:**4596–4603.
4. **Chen, W., J. A. Harp, and A. G. Harmsen.** 1993. Requirements for $CD4^+$ cells and gamma interferon in resolution of established *Cryptosporidium parvum* infection in mice. Infect. Immun. **61:**3928–3932.
5. **Current, W. C., and L. S. Garcia.** 1991. Cryptosporidiosis. Clin. Microbiol. Rev. **4:**325–358.
6. **Davies, M. D., and D. M. Parrott.** 1981. Preparation and purification of lymphocytes from the epithelium and lamina propria of murine small intestine. Gut **22:**481–488.
7. **Dillon, S. B., B. J. Dalton, and T. T. MacDonald.** 1986. Lymphokine production by mitogen and antigen activated mouse intra-epithelial lymphocytes. Cell. Immunol. **103:**326–338.

8. **Dimier, I. H., and D. T. Bout.** 1993. Rat intestinal epithelial cell line IEC-6 is activated by recombinant interferon-γ to inhibit replication of the coccidian *Toxoplasma gondii*. Eur. J. Immunol. **23:**981–983.
9. **Findly, R. C., S. J. Roberts, and A. C. Hayday.** 1993. Dynamic response of murine gut intraepithelial T cells after infection by the coccidian parasite *Eimeria*. Eur. J. Immunol. **23:**2557–2564.
10. **Goodgame, R. W., K. Kimball, C.-N. Ou, A. C. White, R. M. Genta, C. H. Lifshitz, and C. N. Chappell.** 1995. Intestinal function and injury in acquired immunodeficiency syndrome-related cryptosporidiosis. Gastroenterology **108:**1075–1082.
11. **Guy-Grand, D. M., M. Malassis-Seris, C. Briottet, and P. Vassali.** 1991. Cytotoxic differentiation of mouse gut thymus-dependent and -independent intraepithelial T lymphocytes is induced locally: correlation between functional assays, presence of perforin and granzyme transcripts, and cytoplasmic granules. J. Exp. Med. **173:**1549–1552.
12. **Heine, J., H. W. Moon, and D. B. Woodmansee.** 1984. Persistent cryptosporidiosis infection in congenitally athymic (nude) mice. Infect. Immun. **43:** 856–859.
13. **Iseki, M.** 1986. Two species of *Cryptosporidium* naturally infecting the house rat, *Rattus norvegicus*. Jpn. J. Parasitol. **35:**521–526.
14. **Kogut, M. H., and C. Lange.** 1989. Interferon-γ-mediated inhibition of the development of *Eimeria tenella* in cultured cells. J. Parasitol. **75:**313–317.
15. **McDonald, V., and G. B. Bancroft.** 1994. Mechanisms of innate and acquired immunity in SCID mice infected with *Cryptosporidium parvum*. Parasite Immunol. **16:**315–320.
16. **McDonald, V., R. Deer, S. Uni, M. Iseki, and G. J. Bancroft.** 1992. Immune responses to *Cryptosporidium muris* and *Cryptosporidium parvum* in adult immunocompetent or immunocompromised (nude and SCID) mice. Infect. Immun. **60:**3325–3331.
17. **McDonald, V., H. A. Robinson, J. P. Kelly, and G. J. Bancroft.** 1994. *Cryptosporidium muris* in adult mice: adoptive transfer of immunity and protective roles of CD4 versus CD8 cells. Infect. Immun. **62:**2289–2294.
18. **McGhee, J. R., J. Mesteky, M. T. Dertzbaugh, and J. Eldridge.** 1992. The mucosal immune system: from fundamental concepts to vaccine development. Vaccine **10:**75–88.
19. **Mead, J. R., M. J. Arrowood, M. C. Healey, and R. S. Sidwell.** 1991. Cryptosporidial infections in SCID mice reconstituted with human or murine lympocytes. J. Protozool. **38:**59S–61S.
20. **Perryman, L. A., P. H. Mason, and C. E. Chrisp.** 1994. Effect of spleen cell populations in resolution of *Cryptosporidium parvum* infection in SCID mice. Infect. Immun. **62:**1474–1477.
21. **Reiman, J., and A. Rudolphi.** 1995. Co-expression of CD8α in $CD4^+$ T cell receptor $\alpha\beta^+$ T cells migrating into the murine small intestine epithelial layer. Eur. J. Immunol. **25:**1580–1585.
22. **Sim, G.-K.** 1995. Intraepithelial lymphocytes and the immune system. Adv. Immunol. **58:**297–343.
23. **Sydora, B. C., P. F. Mixter, H. R. Holcombe, P. Eghtesady, K. Williams, M. C. Amaral, A. Nel, and M. Kronenberg.** 1993. Intestinal intra-epithelial lymphocytes are activated and cytolytic but do not proliferate as well as other T cells in response to mitogenic signals. J. Immunol. **150:**2179–2191.
24. **Tilley, M., V. McDonald, and G. J. Bancroft.** Resolution of cryptosporidial infection in mice correlates with parasite-specific lymphocyte proliferation associated with both T_h1 and T_h2 cytokine secretion. Parasite Immunol. **17:**459–464.
25. **Tyzzer, E. E.** 1907. A protozoan found in the peptic glands of the common mouse. Proc. Soc. Exp. Biol. Med. **5:**12–13.
26. **Ungar, B. L. P., J. A. Burris, C. A. Quinn, and F. D. Finkelman.** 1990. New mouse models for chronic *Cryptosporidium* infection in immunodeficient hosts. Infect. Immun. **58:**961–969.
27. **Ungar, B. L. P., T.-C. Kao, J. L. Burris, and F. D. Finkelman.** 1991. *Cryptosporidium* infection in the adult mouse: independent roles for IFN-γ and $CD4^+$ T lymphocytes in protective immunity. J. Immunol. **147:**1014–1022.
28. **Yamamoto, M., K. Fujihashi, K. W. Beagley, J. R. McGhee, and H. Kiyono.** 1993. Cytokine synthesis by intestinal intraepithelial lymphocytes. J. Immunol. **150:**106–114.

Editor: D. H. Howard