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Immunology

T-cell $\alpha\beta^+$ and $\gamma\delta^+$ deficient mice display abnormal but distinct phenotypes toward a natural, widespread infection of the intestinal epithelium

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ABSTRACT **Vertebrate immune systems contain T cells bearing either $\alpha\beta$ or $\gamma\delta$ T-cell antigen receptors (TCRs). $\alpha\beta$ T cells perform all well-characterized T-cell effector functions, while the biological functions of $\gamma\delta^+$ cells remain unclear. Of particular interest is the role of $\gamma\delta^+$ cells during epithelial infections, since $\gamma\delta^+$ cells are commonly abundant within epithelia. *Eimeria spp.* are intracellular protozoa that infect epithelia of most vertebrates, causing coccidiosis. This study shows that in response to *Eimeria vermiformis*, mice lacking $\alpha\beta$ T cells display defects in protective immunity, while mice lacking $\gamma\delta^+$ cells display exaggerated intestinal damage, apparently due to a failure to regulate the consequences of the $\alpha\beta$ T cell response. An immuno-downregulatory role during infection, and during autoimmune disease, may be a general one for $\gamma\delta^+$ cells.**

Little is known about the development of immunity or the maintenance of tissue integrity during epithelial infection. A unique feature of epithelial immunity is the potential involvement of intraepithelial lymphocytes (IELs), that mostly are T cells (1). Interestingly, IELs are commonly enriched in $\gamma\delta^+$ cells (2–7). Hence, an animal's response to epithelial infection is germane to the biology of $\gamma\delta^+$ cells.

Eimeria spp are protozoan parasites that infect small intestinal epithelial cells of most vertebrates, causing coccidiosis, a severe agricultural problem (8, 9). Infectious oocysts of *Eimeria vermiformis*, a murine pathogen, can be delivered experimentally by the natural route (10), whereupon they infect enterocytes and replicate, undergoing a typical Apicomplexan life cycle, before release as oocysts into the lumen. Infection is marked by transient hyperplasia of the crypts of Lieberkuhn, and villus shortening, common responses of the intestinal mucosa to insult (11). Nevertheless, infection by *E. vermiformis* is relatively mild, without overt diarrhea or bleeding.

The immune response to *E. vermiformis* is manifest in the capacity to attenuate primary infection, and the development of immunity to secondary infection. Strains termed "resistant" (e.g., BALB/c) attenuate oocyst yield and the time over which oocysts are shed (patent period) to a greater degree than "susceptible" strains (e.g., C57.BL/6 or 129) (12–16). Nevertheless, both resistant and susceptible strains are equally immune to secondary challenge (14).

By contrast, athymic nude mice and rats are highly susceptible to primary and secondary infections (17, 18). However, because *nu/nu* animals harbor defects in $\alpha\beta$ T cells, $\gamma\delta$ T cells, and epithelia (the target for infection), these data do not define any one cell type critical to the host response. Nonetheless, a major role for $\alpha\beta^+$ T cells was indicated by other studies, such as those where immunity was conferred on naive mice by transfer of mesenteric lymph node (MLN) $CD4^+$ T cells from infected mice (19, 20). At the same time, the potential involvement of both $\alpha\beta^+$ T cells and $\gamma\delta^+$ cells was suggested by dynamic changes in both cell types during infection (21).

To clarify the involvement of T cells in the anticoccidial response, this study has utilized mice congenitally deficient in, respectively, $\gamma\delta^+$ cells, $\alpha\beta$ T cells, all T cells, or all B cells and T cells. The data show that without $\alpha\beta$ T cells, mice are severely immunocompromised, while without $\gamma\delta^+$ cells, mice display exaggerated mucosal injury, a phenocopy of infection by more virulent strains of pathogen. [Note, while T-cell antigen receptor (TCR)$\alpha-/-$ mice may suffer inherent colitis (22, 23), this is distant from and distal to the site targeted by *E. vermiformis*. Moreover, TCR$\alpha-/-$ colitis is induced by pathogens, mostly in older mice (24), whereas our studies used young mice in clean conditions, and colitis was rare in subsequent histological examination.]

MATERIALS AND METHODS

Animals. TCR$\alpha-/-$ (25), TCR$\alpha-/+$, and TCR$\beta\times\delta-/-$ mice (C57.BL/6 background) (bred locally), and TCR$\delta-/-$ (26), RAG2$-/-$ (27), TCR$\beta-/-$, C57.BL/6, C57BL/6 × 129.F2, and 129 mice (from The Jackson Laboratories) were all maintained specific pathogen free (at Yale University), in an accredited facility, on an invariant diet (Hamster chow 3500; Agway, Waverly, NY) with water ad libitum. Mice were 6–10 weeks of age for primary infection.

Parasites and Oocyst Enumeration. *E. vermiformis* (kind gift of K. Todd, University of Illinois) was passaged in mice and oocysts purified and sporulated (14). After microscopically scoring stocks for sporulation, mice were given 10^3 or 10^2 oocysts in 100 μl of water by oral gavage. During infection, mice were usually maintained singly on racks above sterile sand, from which feces were collected every day. Oocysts were counted on McMaster chambers after salt flotation, from day 5 postinfection (D5PI) until no oocysts were detected.

Gut Lymphocyte Preparation and Transfer. IELs were prepared as described (21). From uninfected 129, C57.BL/6, TCR$\alpha-/-$, and TCR$\delta-/-$ mice, comparable numbers of IELs were reproducibly harvested: >98% of $CD3^+$ IELs in TCR$\alpha-/-$ mice were $\gamma\delta^+$; conversely TCR$\delta-/-$ mice contained $\alpha\beta^+$ IELs but no $\gamma\delta^+$ IELs. Furthermore, IELs from TCR$\alpha-/-$ mice and TCR$\delta-/-$ mice displayed surface profiles—e.g. $CD8\alpha\alpha(+)$, associated with normal gut IELs (data not shown). Histological examination revealed the area of the small intestine targeted by *E. vermiformis* to be morpho-

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Abbreviations: IEL, intraepithelial lymphocyte; TCR, T-cell antigen receptor; MLN, mesenteric lymph node; D*n*PI, day *n* postinfection; FCS, fetal calf serum; FACS, fluorecence-activated cell sorter.

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Table 1. Infection of TCRα KO mice: Comparison with heterozygous littermates and parental strains

Exp.	Mouse strain	Primary infection: No. mice/ group	Primary infection: Oocyst production (×10⁶ ± SE)	Primary infection: Patent period (days)	Secondary infection: No. mice/ group	Secondary infection: Days after primary infection	Secondary infection: Oocyst production (×10⁶ ± SE)	Secondary infection: Patent period (days)	% of primary α −/− control*
1	α−/−	4	96.6 ± 13.4[a]	12.0 ± 0.5	2	40	17.5 ± 7.9[a]	9.0 ± 0.0	17.7
	Littermate+/−	4	23.0 ± 10.9[b]	4.5 ± 0.5	2	40	0.0 ± 0.0[b]	0.0 ± 0.0	
	129	4	26.6 ± 5.3[b]	7.3 ± 0.5	2	40	0.03 ± 0.0[b]	4.0 ± 0.0	
	BALB/c	4	0.7 ± 0.0[c]	3.3 ± 0.3	2	40	0.0 ± 0.0[b]	0.0 ± 0.0	
	α−/−				1	NA	99.0†	12†	
2	α−/−	4	0.28†	10†	4	29	0.5†	4†	26.7
	Littermate+/−	4	0.12†	6†	4	29	0.0†	0†	
	129	4	0.19†	7†	4	29	0.0†	0†	
	BALB/c	4	0.13†	7†	4	29	0.0†	0†	
	α −/−				8	NA	1.8†	4†	
3	α−/−	4	0.28†	10†	4	29 + 127	56.7†	7†	69.2
	Littermate+/−	4	0.28†	6†	4	29 + 127	0.0†	0†	
	α−/−				4	NA	81.9†	9†	
4	α−/−	4	72.8 ± 4.4[a]	13.0 + 0.0	3	30	56.39 ± 23.2[a]	13.8 ± 0.8	—‡
	α+/−	5	12.0 ± 3.0[b]	8.0 + 0.8	5	30	0.0[b]	0	

Results are expressed as mean + SEM. Within columns and experiments, values not followed by a common superscript letter are significantly different ($P < 0.05$). NA, not applicable.

*Calculated as percent of yield from same genotype mice given a primary infection in parallel with the test secondary infection.

†Oocyst output calculated from animals housed in group cages; therefore SE and statistics are not available in these experiments.

‡Percent yield compared with primary could not be estimated because no primary infection was run in parallel.

logically indistinguishable in all mice utilised. For adoptive transfer of IELs, cells were given intraperitoneally in phosphate buffered saline pH 7.2 (PBS) plus 2% fetal calf serum (FCS).

Adoptive Transfer of MLN Cells. Cells were harvested from the MLNs of TCRα+/− mice, D3 postsecondary infection by affinity chromatography using IsoCell glass bead columns (Pierce) to which were conjugated goat anti-mouse immunoglobulin, anti-mouse major histocompatibility complex class II, and anti-mouse CD8. Fluorecence-activated cell sorter (FACS) analysis of the flow-through cells showed them to be >96% $CD4^+$, 10^7 of which were given i.v. (in PBS plus 2% FCS) 24 hr before infection.

Cell Staining. IELs (10^6) in 100 μl of staining buffer (1× PBS/2% FCS/0.01% sodium azide) were reacted for 30 min on ice in 96-well plates with anti-γδ (GL-3)-phycoerythrin (PE); anti-CD3(2C11)-fluorescence isothiocyanate (FITC); and anti-αβ(H-57)-PE. PE-conjugated antibodies were used at 1:100 and FITC antibodies at 1:200. Cells were washed 3 times and fixed in 1% paraformaldahyde before analysis on a FACScan (Becton Dickinson) with LYSIS software. For double-staining, antibodies (PharMingen) were added together. The negative staining baseline was determined by use of directly conjugated (PE or FITC) normal hamster immunoglobulin.

Fecal Hemoglobin Detection. One day's fecal output was suspended overnight at 4°C in 7 ml sterile water with 1 mM phenylmethylsulfonyl fluoride. Solid material was spun out, and the supernatant photographed, and mixed 3:1 with 3× SDS/PAGE sample buffer containing fresh dithiothreitol. Aliquots were electrophoresed through SDS/PAGE, electroblotted (Bio-Rad) to nitrocellulose membranes, and reacted with rabbit antiserum to mouse hemoglobin (Research Plus, Bayonne, NJ). The membranes were probed with horse radish peroxidase-conjugated goat anti-rabbit IgG, washed, and developed using chemiluminescence-based reagents (ECL; Amersham) in the linear range according to the manufacturer's instructions.

Histology. Freshly explanted gut pieces were fixed in 10% formal saline overnight and paraffin-embedded. Sections (4 μm) were applied to poly-L-lysine-treated slides and stained with hemotoxylin and eosin.

Examination *en face* of the Mucosa. Freshly explanted small intestine was opened longitudinally, placed in PBS plus 2% FCS, and photographed using a dissecting microscope with overhead camera port (Nikon 35 mm, Kodak T-160 Ektachome film).

Statistical Analysis. Mann–Whitney *U* test was used, and statistical significance accepted at $P < 0.05$.

RESULTS

Primary and Secondary Infection of TCR α−/− Mice. Infections were compared in TCRα−/− and TCRα+/− littermates, and in the parental strains, 129 (susceptible), and BALB/c (resistant). Data in Table 1 are from four independent experiments using 60 mice that commonly were housed and studied singly. Variability in oocyst yields between experiments reflects different doses and stocks as inocula. But because each experiment was rigorously internally controlled, clear conclusions could be drawn. Notably, TCRα−/− mice

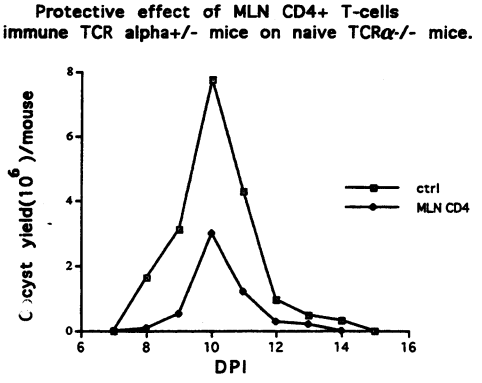

FIG. 1. Effect of adoptively transferred $CD4^+$ MLN cells on oocyst output of infected TCRα−/− mice. Open squares show daily oocyst output of control TCRα−/− mice; filled squares show output from TCRα−/− mice receiving 10^7 $CD4^+$ MLN cells from immune TCRα+/− donors 24 hr prior to challenge.

Table 2. Infection of TCR δ KO mice: Comparison with parent strains

		Primary infection				Secondary infection*		
Exp.	Mouse strain	No. mice/group	Dose	Oocyst production ($\times 10^6 \pm$ SE)	Patent period (days + SE)	Dose	Oocyst production ($\times 10^6 \pm$ SE)	Patent period (days + SE)
1	δ −/−	4	1000	10.81 ± 0.97[a]	7.5 ± 0.0	ND		
	C57 B1/6	4	1000	16.58 ± 4.29[a]	9.5 ± 0.5	ND		
	129	4	1000	9.46 ± 0.41[a]	7.5 ± 0.5	ND		
2	δ −/−	3	1000	5.57 ± 1.0[a]	8.7 ± 0.3	1000	0.0 ± 0.0[a]	0.0 ± 0.0
	C57 B1/6	3	1000	11.06 ± 2.03[a]	8.7 ± 0.3	1000	0.0 ± 0.0[a]	0.0 ± 0.0
	129	3	1000	12.66 ± 2.54[a]	7.0 ± 0.5	1000	0.0 ± 0.0[a]	0.0 ± 0.0
	δ −/−	4	100	1.50 ± 0.80[b]	8.0 ± 0.0	1000	0.0 ± 0.0[a]	0.0 ± 0.0
	C57 B1/6	4	100	2.13 ± 1.12[b]	7.3 ± 0.3	1000	0.0 ± 0.0[a]	0.0 ± 0.0
	129	4	100	7.30 ± 1.43[c]	6.5 ± 0.3	1000	0.0 ± 0.0[a]	0.0 ± 0.0
	C57 B1/6	4	ND			1000	12.57 ± 1.76	7.7 ± 0.5

Results are expressed as mean + SEM. Within columns and experiments, values not followed by a common superscript letter are significantly different ($P < 0.05$). ND, not done.
*For secondary infection, mice were challenged 30 days post-primary infection.

showed oocyst yields and patent periods always as high and commonly higher than primary infections of any other mice tested, and failed to display full protective immunity, whether rechallenged 1 month later with 100 oocysts (experiment 2); rechallenged 1 month later with 1000 oocysts (experiment 4); rechallenged 40 days later with 1000 oocysts (experiment 1); or rechallenged twice with 1000 oocysts, first after 29 days, and second after a further 127 days (Table 1). Nonetheless, oocyst yields were never as high as those from naive TCRα−/− mice infected in parallel, although the range (17.7–69.2%) was broad (see *Discussion*).

Adoptive transfer was used to test whether αβ cells could complement the TCRα−/− defect (Fig. 1). MLN $CD4^+$ T cells were enriched by negative selection from TCRα+/− mice at D3 post-secondary infection (D43 post-primary infection), and following phenotypic characterization of an aliquot by FACS, were transferred (10^7 cells) i.v. to nonirradiated, naive TCRα−/− littermates. After infection 24 hr later, recipient mice showed a 70% reduction of oocyst yield, compared with mice receiving sham inocula. These data compare well with a 50% reduction in oocyst yield reported after 10^7 $CD4^+$ MLNs were transferred from infected to irradiated naive NIH mice (19). Thus, the defect in the primary response shown by TCRα−/− mice can be complemented in large part by provision of $CD4^+$ $\alpha\beta^+$ T cells.

Primary and Secondary Infections of TCRδ−/− Mice. A comparison of TCRδ−/− mice, and the parental strains, C57.BL/6 and 129, was made in two experiments using 37 mice, caged singly (Table 2). Again, there was variability between experiments, and 129 mice showed increased susceptibility at lower doses of infection. Nonetheless, total oocyst yields from TCRδ−/− mice did not differ significantly from those of the parental strains, and TCRδ−/− mice displayed full immunity toward 10^3 oocysts, with which they were rechallenged on D30PI (Table 2).

Changes in Mucosal Morphology in Infected TCRδ−/− Mice. To enumerate oocyst production, fecal pellets collected from individually housed mice were soaked overnight in water. D8PI TCRδ−/− supernatants were commonly red-brown, and always darker than those of other strains (100% penetrance, $n = 37$ mice). D8PI corresponds with peak morphological changes in the intestinal mucosa (13), and with significant mobilization of the systemic immune response, as judged by the expansion of *Eimeria*-reactive αβ T cells in the MLNs (19, 21).

While several possibilities (e.g., altered bile excretory products) existed for the red-brown material, exaggerated blood loss seemed a likely explanation. Variability in color (red/brown) would be expected, given that oxygenation will vary. Hemoccult tests were invariably positive on D8PI fecal extracts of TCRδ−/− mice (data not shown), supporting the interpretation, but hemoccult tests are a plus/minus measure for blood (28), and also scored positive for other D8PI fecal supernatants that were not overtly discolored. However, several other results indicated that TCRδ−/− mice suffered exaggerated blood loss.

First, D8PI TCRδ−/− fecal supernatants showed Western blot reactivity with a serum against mouse hemoglobin. While it was to be expected that blood in mouse fecal suspensions would be partially degraded (28), several protein bands of ≈17 kDa were detected in common in fecal supernatants from four individual TCRδ−/− mice (Fig. 2, lanes 2–5), and in a control mixture of mouse blood and fecal supernatants of uninfected mice (Fig. 2, lanes 1 and 6). By contrast, little immunoreactivity was shown by fecal supernatants from D8PI C57.BL/6 mice (Fig. 2, lanes 7–10).

Second, by microscopic observation *en face* of the D8PI mucosa, it was common to detect in TCRδ−/− mice, focal red areas within villi (Fig. 3*b*, arrows), that commonly discharged onto the lumenal surface (Fig. 3 *c* and *d*). Such was not seen in C57.BL/6 (Fig. 3*a*) or TCRα−/− mice. Infected intestines of all strains showed swaths of parasite-associated damage separated by normal areas. The hemorrhages characteristic of TCRδ−/− mice occurred only in areas targeted by the parasite.

Next, blocks of D8PI TCRδ−/− intestine dissected from areas of visible bleeding showed overt blood cell extravasation (arrows in Fig. 4*a*), and conspicuous villus tip damage (arrows in Fig. 4*b*). Vessel congestion, shown by arrows in an adjacent villus (Fig. 4*b*), commonly reflects cytokine-induced capillary dilitation, and frequently precedes red cell extravasation. These features of villus damage were supple-

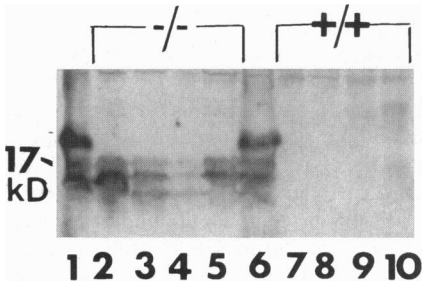

FIG. 2. Western blot of supernatants of D8PI fecal output from four individual TCRδ−/− mice (−/−; lanes 2–5), and four individual C57.BL/6 mice (+/+; lanes 7–10). Lanes 1 and 6, aliquots of mouse blood, mixed overnight with fecal supernatant from uninfected C57.BL/6 mice. Size marker comigration is indicated.

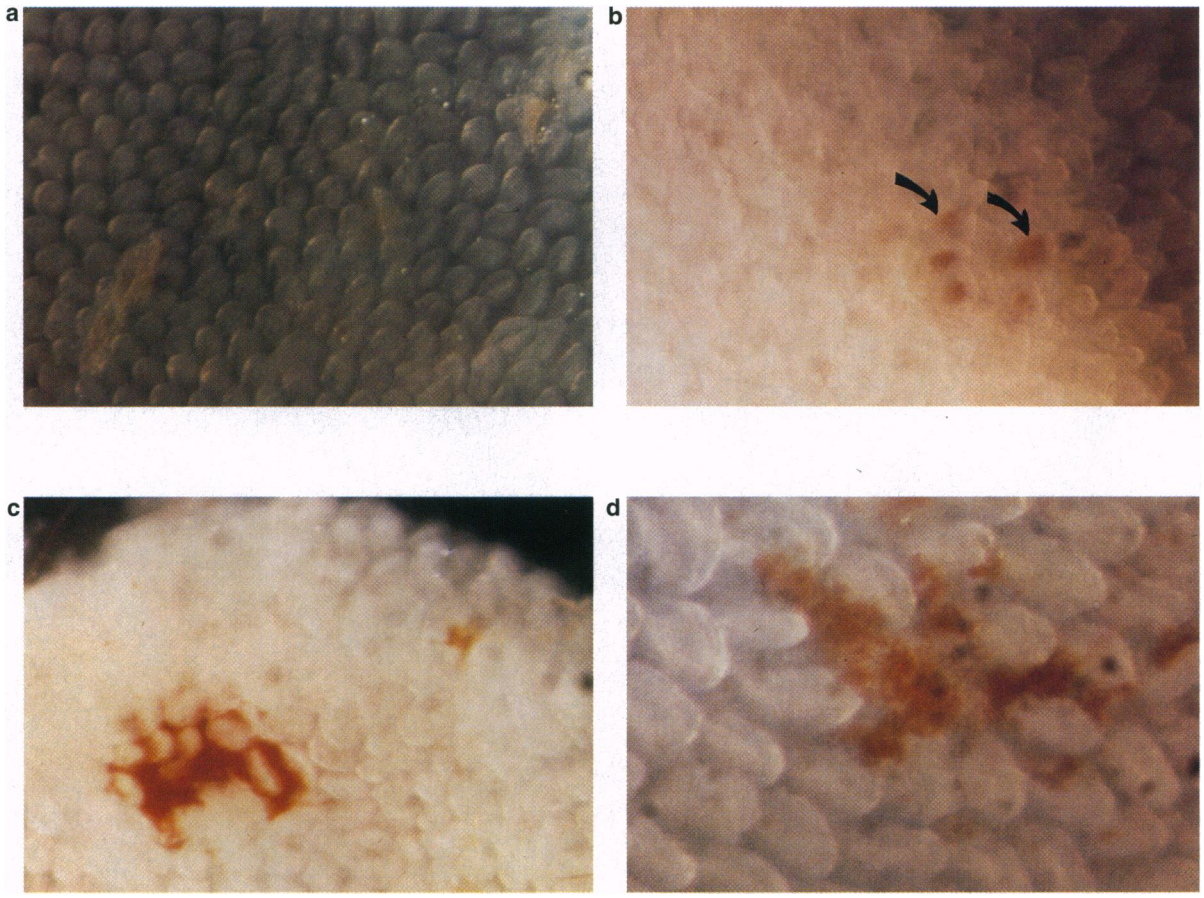

FIG. 3. Examination *en face* of the small intestines of mice used in this study. (*a*) Infected C57.BL/6, D8PI. (*b–d*) TCRδ−/−, D8PI. Arrows in *b* denote conspicuous reddening within villi.

mental to crypt hyperplasia, villus shortening, and increased numbers of lamina propria immunocytes, all of which are characteristic of *Eimeria* infections (13). A neutrophil infiltrate is marked with an open arrow in Fig. 4*a*. Scores of sections cut from infected C57.BL/6, 129, and TCRα−/− mice (Fig. 5*a*) commonly showed heavy parasitic infestation (arrows), but red cell extravasation was negligible compared with TCRδ−/− mice (Fig. 5 and data not shown).

Bleeding in TCRδ−/− Mice Is Immune-Mediated and Can Be Ameliorated by Provision of IELs. Discoloration of fecal supernatants was used to assess mucosal injury in other immunodeficient strains. Even when susceptibility to infection was extreme (e.g., in primary or secondary infections of RAG2−/− mice, TCR(βxδ)−/− mice, TCRβ−/−, or TCRα−/− mice), supernatants (Fig. 6, tubes 2–4) were comparable to those of TCR(β×δ)−/− mice (tube 5), and lighter than those of TCRδ−/− mice (tubes 1, 6) infected in parallel. The failure of RAG−/− mice and TCR(β×δ)−/− mice to score in this assay suggested that the exaggerated bleeding in TCRδ−/− mice required αβ T cells (discussed below).

To determine whether the TCRδ−/− phenotype could be rescued, 2×10^6 IELs from (C57.BL/6 × 129)F_2 mice were transferred i.p. to TCRδ−/− recipients that were infected with 1000 oocysts of *E. vermiformis* 2 days later. This inoculum completely ameliorated the phenotype (Fig. 6, tube 7), compared with a TCRδ−/− mouse mock-reconstituted (500 μl of PBS plus 2% FCS), and infected in parallel (tube 8). The IEL dose conferring rescue varied, as is commonly the case for adoptive transfers: in another experiment, rescue was observed using 7.5×10^6 cells (tube 9), compared with either unmanipulated (tube 12) or mock-reconstituted TCRδ−/− mice (tubes 10 and 11).

DISCUSSION

Data presented here establish the critical role of αβ T cells in protecting the host, both in primary and in subsequent infections by the coccidian, *E. vermiformis*. Conversely, γδ T cells appear to be important regulators of the host response, since TCRδ−/− mice display heightened blood loss, manifest in discoloration of fecal supernatants, high reactivity of fecal supernatants to anti-mouse hemoglobin, hemorrhage evident in *en face* examination of the intact mucosa, and villus tip damage and red cell extravasation, revealed in histologic sections.

The findings relate to traditional measures of pathogen virulence and host-responsiveness. Past studies of *Eimeria spp.* in chickens have shown discrepancies between measures of oocyst yield, lesion scores, and reduced weight gain (see ref. 16). We can now propose that those different assays may reflect the effects of different host cells: oocyst yield may directly reflect αβ T-cell function, whereas lesion scores and weight gain may reflect γδ-cell function.

Neither RAG2−/− mice nor TCR(βxδ)−/− mice showed discolored fecal supernatants, indicating that the exaggerated bleeding in TCRδ−/− mice is mediated by responding αβ T cells, and/or cells activated by them, that under normal circumstances would be regulated by γδ cells. Nevertheless, TCRδ−/− mice did not show overt pathology on reinfection, despite the fact that secondary immune responses are rapid and effective. Thus the immuno-pathology normally regulated by $\gamma\delta^+$ cells is most likely provoked by the prolonged response to sustained antigenic load, characteristic of primary infection. Indeed, MLN expansion and local inflammation during secondary responses are less than in primary infection. Overt bleeding was also reported upon

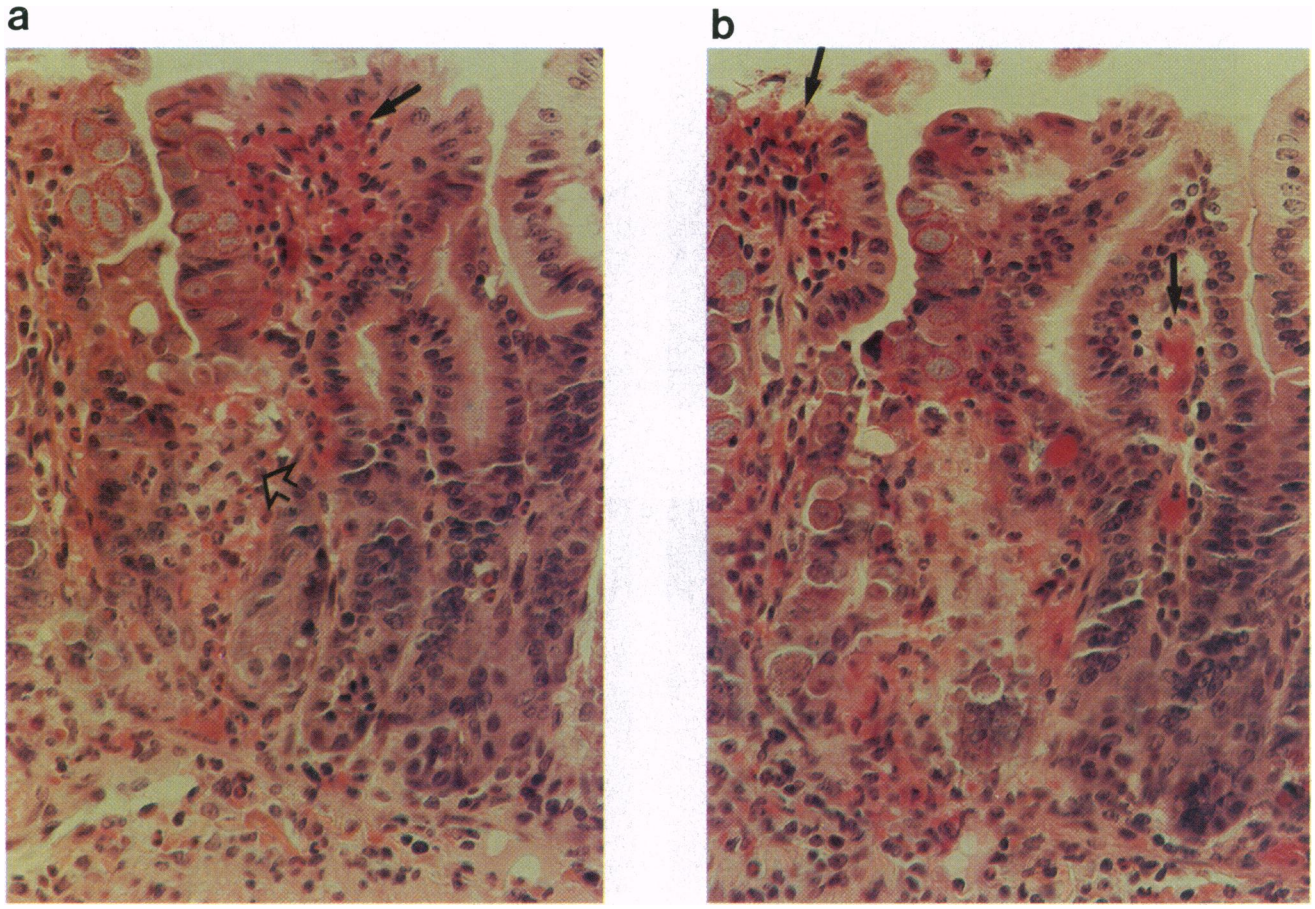

FIG. 4. Haematoxylin and eosin-stained sections of TCRδ−/− small intestine, D8PI. Parasites are abundant and were associated with red blood cell extravasation (arrows in *a*), damaged villi (arrows in *b*), and capillary congestion (arrows in *b*). Open arrow in *a* denotes a neutrophil infiltration.

tertiary infection of nude mice with *Eimeria falciformis* (17). However, because a different species of *Eimeria* was used, and because of the uncertain effects of the epithelial defect in nude mice, it is difficult to compare that bleeding with observations made here.

An immuno-regulatory role may be a general one for γδ cells: skin-associated γδ T cells prevented disruptive infiltration of the epidermis by αβ T cells targeting Langerhans cells (29); mice depleted of γδ T cells by antibody administration, and infected with *Listeria* via a single testicle,

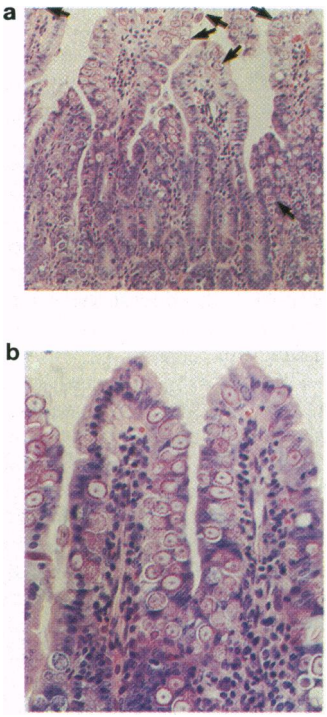

FIG. 5. Haematoxylin and eosin-stained sections of TCRα−/− small intestine, D8PI. Mature parasites are abundant (arrows), associated with damaged villi, but there is negligible red cell extravasation, capillary congestion, or villus tip breakage.

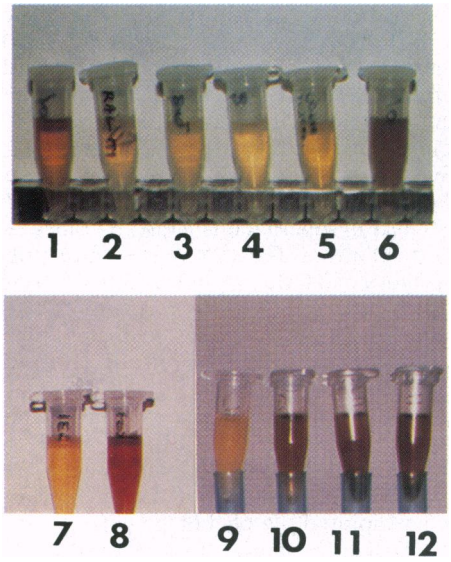

FIG. 6. Supernatants of fecal pellets collected from individual mice on D8PI. Tubes: 1, 6, and 12, TCRδ−/− unmanipulated; 2, RAG2−/−; 3, TCRβ×δ−/−; 4, TCRβ−/−; 5, TCRβ×δ+/− "double heterozygotes;" 7 and 9, TCRδ−/− adoptively transferred with 2×10^6 or 7.5×10^6 IELs, respectively; 8, 10, and 11, TCRδ−/− mock adoptively transferred.

developed increased inflammatory injury in the contralateral, uninfected testicle (30); and the $\alpha\beta$ T-cell-dependent liver granulomas that help sequester *Listeria monocytogenes* in mice infected i.p. were larger and "abcess like" in the absence of $\gamma\delta^+$ cells (31, 32). It will be important to resolve the ligand specificity of the $\gamma\delta$ TCR that underlies these effects.

Several mechanisms may be envisaged by which $\gamma\delta$ cells might offset the actions of other responding cells—e.g., the release of cytokines such as keratinocyte growth factor, that can repair local tissues (33); the release of inhibitors of effector cytokines, analogous to the competitive inhibition of IL1 (34); the release of interleukin 4, interleukin 10, or transforming growth factor β, that could redirect local immunological activity; and the direct interaction of $\gamma\delta$ cells with enterocytes, via an integrin, $\alpha_{IEL}\beta_7$ (35–38) that binds E-cadherin on enterocytes (39). Such an interaction might underlie reported effects of $\gamma\delta$ cells on epithelial turnover and differentiation (40).

Clearly, the amelioration of immunopathology attributed in this paper to $\gamma\delta$ T cells may not be unique to $\gamma\delta$ T cells. Nonetheless, the reproducible phenotype of the TCR$\delta-/-$ mice suggests that $\gamma\delta$ T-cell deficiency is a significant burden *vis-a-vis* these effects. Such effects may have direct relevance to human disease. In coeliac disease, an allergy to gluten in which gut $\gamma\delta$ cells are expanded (41), the ratio of $\gamma\delta$ T cells to $\alpha\beta$ T cells is highest during "silent coeliac disease" marked by mild pathology. Possibly this phase marks the downregulation of activated $\alpha\beta$ T cells by expanded $\gamma\delta$ T cells. Likewise, frequent reports of $\gamma\delta$ cells in autoimmune lesions—e.g. those associated with lupus and multiple sclerosis—may reflect a downregulatory, rather than an effector, T-cell infiltrate.

Immune-protective functions have also been proposed for $\gamma\delta$ T cells (42). $\gamma\delta$ cells may provide an immune-protective function in coccidiosis, since data presented in Table 1 show that TCR$\alpha-/-$ mice (that contain $\gamma\delta$ T cells) are less susceptible to secondary infection than to primary infection. The "resistance" of such TCR$\alpha-/-$ mice was variable, possibly reflecting variable numbers of $\gamma\delta$ T cells in TCR$\alpha-/-$ mice (43). Likewise, an auxiliary function for $\gamma\delta^+$ T cells has been noted in immunity to chronic *Mycobacterium bovis* (44) and acute *L. monocytogenes* (31, 32) infections. Nonetheless, it is striking that in no case does a TCR$\alpha-/-$ mouse develop full and rapid immunity. Given the increasing evidence for diversity in TCR$\gamma\delta$ (45), the problem may lie in the failure of $\gamma\delta$ cells to recognize foreign antigens as presented as peptides on major histocompatibility complex, thus limiting the capacity of $\gamma\delta$ cell to sustain a conventional protective immune response (46). This failure may be heightened by the particular physiology and anatomy of $\gamma\delta$ cells (46), emphasizing the importance of using natural routes of infection and natural doses of pathogen, when studying $\gamma\delta$ T-cell biology.

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