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T-helper subset function in the gut of rats: differential stimulation of eosinophils, mucosal mast cells and antibody-forming cells by $OX8^-$ $OX22^-$ and $OX8^-$ $OX22^+$ cells

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SUMMARY

Thoracic duct lymphocytes (TDL) collected 3 days after infection of rats with *Trichinella spiralis* (TS) and adoptively transferred into normal, uninfected recipients, increased the numbers of both mucosal mast cells (MMC) and eosinophils (EOS) in the intestine. The $CD4^+$ T-helper cell population was separated into two subsets ($OX22^+$ and $OX22^-$) using OX22 monoclonal antibody (mAb) and panning techniques. After adoptive transfer of these T-helper subsets i.v., rats were challenged with TS 24 hr later. The intestine of recipient rats was examined histologically at intervals from Day 3 to Day 21. On Day 9 after transfer, $OX22^+$ T helpers induced a substantial mastocytosis [94 ± 3, mean $\pm$ SE/villus crypt unit (VCU)], whereas the $OX22^-$ T-helper subset increased resident EOS numbers (60 ± 2/VCU) compared to the challenge control (18 ± 1 MMC, 27 ± 1 EOS/VCU). The time of peak eosinophilia was advanced by 3–6 days for recipients of $OX22^-$ cells and that of mast cells by 9–12 days for recipients of $OX22^+$ cells. The recipients of $OX22^-$, but not $OX22^+$, cells also showed a large increase in the numbers of B cells in the spleen and mesenteric lymph node (MLN) secreting antibody against adult TS. Recipients of $OX22^-$ cells displayed an even increase in EOS throughout the villi, lamina propria (LP) and muscularis, whereas in $OX22^+$ cell recipients mast cells were only present in the lower villus and the epithelium just above the crypt as well as the muscularis layer. Only the $CD4^+$ $OX22^-$ cell subset conferred protection against TS in the intestine. We conclude that the $OX22^+$ and $OX22^-$ T-helper cells exert distinctive effects in the intestine on MMC and EOS. Because protection was established in the presence of an $OX22^-$ T-helper-induced eosinophilia but without a concurrent mastocytosis, the results suggest that MMC are probably not involved in expulsion of TS to terminate the primary infection.

INTRODUCTION

The existence of two functionally distinct subsets (Th1 and Th2) of T-helper cells has been well established (Mosmann *et al.*, 1986; Cher & Mosmann, 1987). In mice, the Th2 subset has been shown to produce interleukins (IL) 4 and 5 and to stimulate production of antibodies of immunoglobulin A (IgA), IgG1 and IgE isotypes. The Th1 subset has been shown to produce lymphotoxin, IL-2, granulocyte–macrophage colony-stimulating factor (GM-CSF), to participate in delayed-type hypersensitivity (DTH) reactions and to stimulate IgG2a and IgG2b production (Mosmann *et al.*, 1986; Cher & Mosmann, 1987; Woods *et al.*, 1987). No surface antigenic markers have yet been defined that distinguish Th1 and Th2 in mice and their separation is based on lymphokine secretion profile. Similar functional divisions among T-helper cells have been demonstrated in humans (Dalchau, Kirkley & Fabre, 1980; Reinherz *et al.*, 1982; Clement, Yamashita & Martin, 1988), although Th1 and Th2 may not exist as such.

In rats, two subsets of T-helper cells have been recognized since the generation of monoclonal antibody (mAb) MRC OX22 (Spickett *et al.*, 1983). This mAb antibody reacts with rat leucocyte common antigen (LCA) expressed in high molecular weight (MW) form (Powrie & Mason, 1988). OX22 divides T-helper cells into two functionally distinct subsets; the $OX22^+$ T-helper cells mediate graft-versus-host alloreactivity, whereas $OX2^-$ T-helper cells provide help for B cells (Spickett *et al.*, 1983; Powie & Mason, 1988). Recently, Powrie & Mason (1989)

Abbreviations: EOS, eosinophils; FIPA, filter immunoplaque assay; LCA, leucocyte common antigen; LP, lamina propria; ML, muscle larvae; MLN, mesenteric lymph node; MMC, mucosal mast cell; PFC, plaque-forming cell; TDL, thoracic duct lymphocyte; TS, *Trichinella spiralis*; VCU, villus crypt unit.

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have suggested that the $OX22^+$ cells are the precursors of $OX22^-$ cells. The $OX22^-$ cell subset also transfers protection against *Trichinella spiralis* (TS) infections (Korenaga *et al.*, 1989) and migrates preferentially to the intestinal epithelium by comparison with $OX22^+$ cells (Wang *et al.*, 1990). However, LCA is lost during cell activation and cells losing LCA are not believed to regain it. Therefore, it is not clear whether the $CD4^+$ cell division based on the OX22 marker corresponds to Th1 and Th2 subsets in rats; the loss of the high MW form of LCA during the process of cell activation could indicate that OX22 expression does not correlate with specific T-cell subset distribution in this species. Nor is it clear that OX22 is lost by all cells undergoing activation *in vivo*.

Intestinal nematode infections induce characteristic immunopathological changes that include eosinopoiesis and mucosal mastocytosis as well as elevations of IgE and IgG1 (Despommier, Weisbroth & Fass, 1974; Rothwell, 1975; Dessein *et al.*, 1981; Woodbury *et al.*, 1984; Appleton, Schain & McGregor, 1988). The increase in both mucosal mast cells (MMC) (Haig *et al.*, 1982; Guy-Grand *et al.*, 1984) and intestinal eosinophils (EOS) (Ruitenberg *et al.*, 1977) is known to be T-cell dependent. EOS are also known to be stimulated by IL-5; therefore the effects of adoptively transferred $OX22^+$ and $OX22^-$ cells on intestinal mast cell or EOS numbers might help delineate T-helper subsets. It is also of interest insofar as both MMC and other inflammatory cells, including EOS, have variously been linked to worm rejection, albeit circumstantially (Rothwell, 1975; Wakelin & Donachie, 1983; Woodbury *et al.*, 1984).

In this report, we examine the effects of adoptively transferred $OX22^+$ and the $OX22^-$ T-helper subpopulations on intestinal EOS and MMC. The results demonstrate that the $OX22^+$ T-helper cells stimulate MMC proliferation whereas the $OX22^-$ T-helper cells can elicit intestinal eosinophilia, activate antigen-specific B cells and confer immunity on recipient rats against TS adult worms in the small intestine.

MATERIALS AND METHODS

Animals and parasites

Male or female AO rats, 6 or more weeks of age, were used in all experiments. Rats were bred at the Baker Institute vivarium and given food and water *ad libitum*. TS was maintained by serial passage in retired breeder rats of PVG or AO strains. The procedures used to isolate larvae to infect rats and to count intestinal worms have been described previously (Bell & McGregor, 1980; Bell, McGregor & Adams, 1982).

Collection of immune thoracic duct lymphocytes (TDL)

Donor rats were infected with 2000 muscle larvae orally on Day 0, on Day 3 of infection the thoracic duct was cannulated and the lymph (TDL) collected for 18–24 hr in heparinized Ringer's solution held at room temperature. Cells were washed with Hanks' balanced salt solution (HBSS) for further separation procedures. In some experiments, unfractionated TDL were infused without further manipulation.

Isolation of T-helper subsets

Affinity chromatography to remove B cells from the TDL cell preparation was undertaken as described elsewhere (Crum & McGregor, 1976). Sephadex G-200 (Pharmacia, Uppsala, Sweden) swollen in distilled water was then activated with cyanogen bromide and coupled with affinity-purified sheep anti-rat $F(ab')_2$ antibodies, made at the Baker Institute. Approximately 100 ml of coupled gel were poured into 50-mm diameter Pharmacia glass columns. After washing off the unbound antibodies, 100 ml of TDL lymphocytes, at a concentration of $1–2 \times 10^7$ ml, were added drop-wise to each column. The columns were washed with 200 ml of HBSS containing 5% foetal bovine serum and the non-adherent surface Ig^- T cells were collected in the effluent. To further isolate T-helper cells a modified panning procedure was used (Chen-Woan, Sajewski & McGregor, 1985), in which the recovered T cells were incubated for 20 min at 4° with mouse anti-rat OX8 mAb (1 : 100 dilution) specific for the cytotoxic/suppressor $CD8^+$ cell subset (Brideau *et al.*, 1980). Cells were then washed twice and were incubated for 30 min at room temperature in Petri dishes ($5–7 \times 10^7$ cells/Petri dish) coated with affinity-purified goat anti-mouse Ig, made at the Baker Institute. The non-adherent $OX8^-$ T-helper cells were poured off after gentle rotation of the Petri dishes. To separate T-helper subsets, the $OX8^-$ cells were then incubated with mouse anti-rat $OX22^+$ mAb (Spickett *et al.*, 1983). A second-step panning was employed to separate the $OX22^+$ adherent cells and the $OX22^-$ non-adherent cells. The OX8 and OX22 mAb were produced at the Baker Institute from the OX8 and OX22 cell lines (a gift from Dr A. Like, University of Massachusetts) with permission from Dr A. Williams (Oxford University, U.K.). All the isolated T-cell populations had >99% viability. The purity of each subset was examined by incubating separated cells with fluoresceinated anti-mouse IgG (H- and L-chain specific; Cappel Laboratories, Malvern, PA) antibody and screening for fluorescence on a FACS IV (Becton-Dickinson, Sunnydale, CA).

Histological examination of the intestinal cellular changes

Immediately after each rat was killed, the small intestine was flushed with saline and a 1·5-cm piece of the anterior small intestine was taken 15 cm from the pylorus. In some experiments, 1·5-cm pieces from the centre of the anterior, middle and the posterior 1/3 of the small intestine were taken. Both ends of the segments of the intestine were tied with surgical silk and 0·2 ml of 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7·4, was injected into the lumen. The tissue was fixed for 6 hr in a vial containing 25 ml of the same fixative and then transferred to 70% ethanol. Standard techniques were used to dehydrate, embed and cut 5 μm sections of the tissue. For examination of EOS and MMC, sections were stained with Alcian blue and counterstained with Lendrum's solution, as described by Newlands, Huntley & Miller (1984). To visualize goblet cells, standard periodic acid schif (PAS) staining was used. Haemotoxylin and eosin (H & E) staining was employed to examine lymphocytes in the epithelium and the lamina propria. Goblet cells appeared dark pink in sections stained with PAS and lymphocytes were dark blue in H & E stained slides. On slides stained with Alcian blue/Lendrum's solution, the blue staining cells were deemed to be MMC, whereas the pink staining cells were EOS as described by Newlands *et al.* (1984). The numbers of cells present were counted in villus crypt units (VCU) and a total of at least 10 VCU was counted for each section.

Determination of anti-adult worm protection

Methods for analysis of immunity against adult worms have been described elsewhere (Bell & McGregor, 1980; Bell *et al.*,

1982). Briefly, donor AO rats were infected with 2000 muscle larvae and immune TDL cells were collected 3 days after infection. T-helper cell subpopulations were isolated and transferred i.v. to AO recipient rats. The recipient rats were then challenged with 1000 muscle larvae 24 hr after cell transfer and adult worm burden in the intestine was assessed at various days after challenge infection. In the cell recipient groups, a significant reduction of adult worm burden compared to the challenge controls was considered evidence of protection.

Quantification of specific antibody-producing cells

The FIPA assay (Wassom, Johnson & Sayles, 1986) was used to demonstrate the presence and expansion of specific anti-TS antibody-producing cells in the spleen and the MLN. A nitrocellulose membrane was assembled in a 48-well chamber and was coated for 45 min at room temperature with crude extracts of TS adult antigen at a concentration of 25 μg/ml, 0·25 ml/well. The unoccupied binding sites of the antigen-coated nitrocellulose membrane were then blocked with PBS containing 3% bovine serum albumin (BSA) for 3 min, washed twice with PBS and then allowed to air dry. The incubated spleen and MLN cells (4×10^6 cells/well) were added to the wells and incubated at 37° for 2 hr in an atmosphere of 5% CO_2. Cells were washed off with PBS and then 0·25 ml rabbit anti-rat Ig $F(ab')_2$, made at the Baker Institute (1:100 dilution), was added to each well. After incubation for 45 min the chamber was washed again and peroxidase-conjugated goat anti-rabbit IgG (Cappel, Malvern, PA) (H- and L-chain specific, 1:800 dilution) was added and incubated for another 30 min. After subsequent washing, 0·25 ml 4-chloro-1-naphthol substrate solution was added to each well and incubated for 15 min at room temperature to develop specific plaques formed on the membrane. After disassembling the chamber and washing the nitrocellulose, the paper was allowed to air dry and the blue plaques were counted at 25× magnification.

Statistics

The significance of differences in mean values between two groups was examined by analysis of variance test and Student–Newman–Keuls test. P values $< 0{\cdot}05$ were considered significant.

RESULTS

Influence of transferred immune TDL on intestinal EOS and MMC

TDL obtained 3 days after TS infection (Day-3 TDL) transfer the capacity to reject TS adult worms from the small intestine of normal recipient rats (Bell, Korenaga & Wang, 1987). To examine the intestinal cellular changes that were induced by these lymphocytes, normal rats were randomized into six groups; on Day 1 rats in Groups 1 and 2 received 1×10^8 normal TDL i.v. while Group 3 and 4 rats received the same number of immune TDL collected from donor rats 3 days after infection. One day after cell transfer, a challenge infection of 1000 ML was given to rats in Groups 2, 4 and 6. Nine days later, a 1·5-cm section of the small intestine 15 cm from the pylorus was taken from each rat in Groups 1 and 2 and processed for histological examination of EOS and MMC. The results showed (Table 1) that in normal rats an average of 13 ± 3 EOS/VCU and $1 \pm 0{\cdot}5$ MMC/VCU were found (see Group 5). Transfer of normal TDL cells did not increase EOS or MMC in the intestine (Group 1). Infection by itself induced eosinophilia and mastocytosis in the gut (Groups 2 and 6). However, immune TDL cells alone could elicit significant intestinal eosinopoiesis and mastocytosis in the absence of an infection in the adoptively transfused recipients (Group 3). No significant increase in peripheral blood EOS counts was observed in rats after transfer of the immune TDL cells (Table 1).

Table 1. Effects of adoptively transferred normal and immune TDL cells on EOS and MMC in the small intestine and on EOS in peripheral blood of recipient rats

	Treatment				
Groups	Cells transferred*	Challenge infection†	EOS/VCU	MMC/VCU	EOS/ml blood ($\times 10^{-5}$)
1	Normal TDL	−	15 ± 2	2 ± 1	$1{\cdot}6 \pm 0{\cdot}4$
2	Normal TDL	+	21 ± 1	8 ± 1	$5{\cdot}3 \pm 2{\cdot}5$
3	Immune TDL	−	32 ± 4	12 ± 3	$4{\cdot}1 \pm 1{\cdot}4$
4	Immune TDL	+	54 ± 6	48 ± 7	$11{\cdot}9 \pm 5{\cdot}3$
5	—	−	13 ± 3	$1 \pm 0{\cdot}5$	$4{\cdot}8 \pm 2{\cdot}9$
6	—	+	25 ± 4	6 ± 1	$8{\cdot}8 \pm 1{\cdot}8$

*TDL cells were injected i.v. into recipient rats at a dose of 1×10^8 cells/rat. Immune TDL cells were collected from donor rats 3 days after infection with 2000 ML.

† 1000 ML were given *per os* as challenge infection.

Blood samples were taken before taking the intestinal tissues diluted in Hinkelman's solution and counted. The small intestines were examined histologically 9 days after challenge infection. For each intestinal sample 10–20 VCU were counted. Data represent means ± 1 SD of four (Groups 1 and 2) or five (the rest of groups) rats per group. Statistic results (by analysis of variance) indicate that for EOS/VCU, significant differences ($P < 0{\cdot}05$) were found in all comparisons between groups except Group 1 versus 5 and Group 2 versus 6. For MMC/VCU, all comparisons were significantly different except Group 2 versus 6; for EOS/ml blood, however, all comparisons were not significantly different ($P > 0{\cdot}05$) except Groups 1 versus 4 and 1 versus 6.

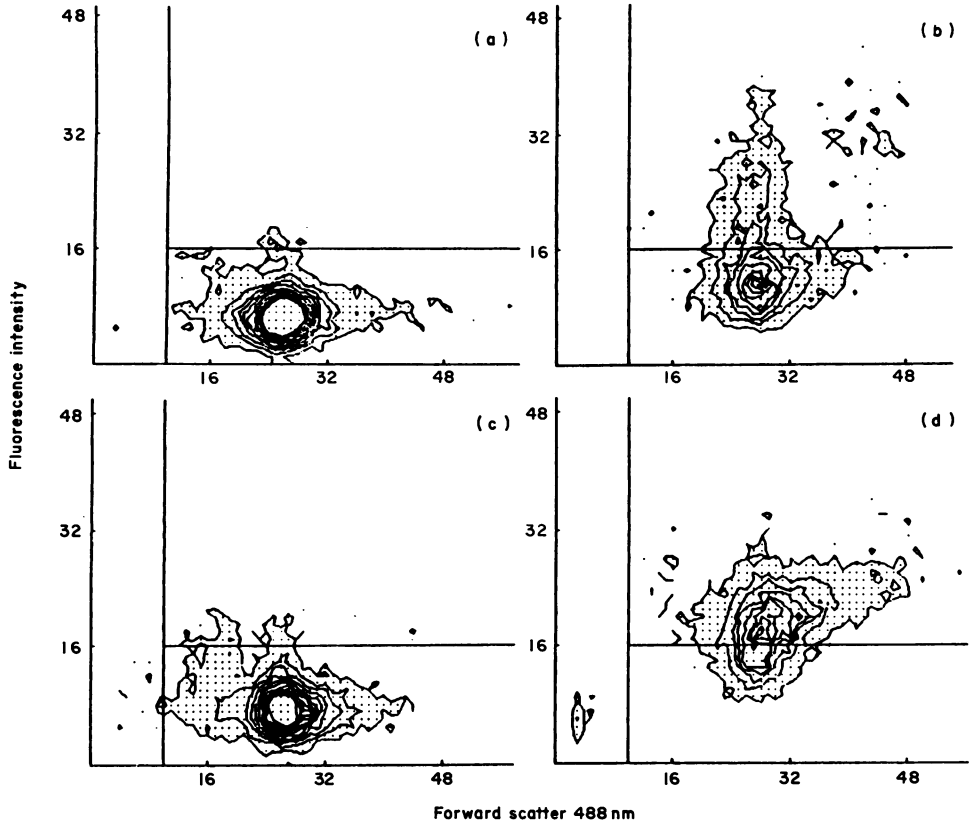

Figure 1. FACS IV analysis of a typical separation procedure employed to obtain OX22$^+$ and OX22$^-$ subsets of rat T-helper cells immunized against TS. (a) The OX8$^-$ cell population stained with mAb OX8 (96% of lymphocytes were negative); (b) the OX8$^-$ population stained with OX22 prior to separation contained 45% OX22$^+$ cells; (c) the OX22$^-$ cell population contained 12% OX22$^+$ cells; (d) the OX22$^+$ population was 81% positive for this marker.

Influence of transfer of isolated Th cell subsets on intestinal cellular changes

To determine which subset(s) of T cells was responsible for eosinopoiesis and mastocytosis, T lymphocytes were isolated by affinity chromatography from Day-3 TDL and the T-helper population (OX8$^-$) was isolated and further divided into two subsets of T-helper (OX8$^-$ OX22$^+$ and OX8$^-$ OX22$^-$) cells by the two-step panning procedure described in the Materials and Methods. The purity of each T-cell population was analysed by FACS; both the T-cell and OX8$^-$ cell subsets were ~96% pure. The OX8$^-$ population contained 35·5% OX22$^+$ cells and the rest were OX22$^-$ T cells (Fig. 1). After panning, the purity of the OX22$^-$ cells was ~90% and that of OX22$^+$ cells 81% (Fig. 1). These cell preparations were injected i.v. as follows: Group 1 rats received 4×10^8 T TDL; Group 2, 2×10^8 OX8$^-$ cells; Group 3, 1×10^8 OX8$^-$, OX22$^+$ cells; Group 4, $2{\cdot}1 \times 10^8$ OX8$^-$, OX22$^-$ cells. The cell dose received by each recipient approximated the output of cells of that phenotype per donor rat during the collection period. Cell recipients and control rats (Group 5) were challenged with 1000 ML orally 24 hr later. The numbers of intestinal EOS, MMC, goblet cells, intraepithelial and intralamina propria lymphocytes were counted 9 days after the challenge infection. For comparison, peripheral blood EOS were also counted.

As summarized in Fig. 2, rats infused with the T TDL or OX8$^-$ T-helper cells showed significant increases in both EOS (twofold) and MMC (threefold) in the intestine compared to the challenge controls. Distinct differences were noted in the inflammatory cell population in the gut in rats receiving OX22$^+$ or OX22$^-$ cells. The OX22$^+$ T-helper cell recipients had 43 ± 3 EOS/VCU and 94 ± 45 MMC/VCU, whereas the OX22$^-$ T-subset recipient had 60 ± 9 EOS and 24 ± 8 MMC/VCU. With the exception of the MMC in OX22$^-$ T-cell recipients and the EOS in OX22$^+$ T-cell recipients, all cell counts were significantly different from the challenge controls. Similar results were derived from several experiments and the data are summarized in Table 2. There were marked differences in the distribution of EOS and MMC in the villi (Fig. 3). EOS occurred throughout the villus/crypt area, whereas MMC were more restricted to the serosal half of the lamina propria. No difference in intestinal goblet cell numbers was found between any cell recipient group and the control. The number of goblet cells/VCU ranged between 21 ± 6 to 34 ± 12/VCU. In all T cell and T-helper subset recipient rats, significantly decreased numbers of intraepithelial lymphocytes (from $2{\cdot}4 \pm 0{\cdot}6$/VCU to $2{\cdot}8 \pm 1{\cdot}4$/VCU) were found compared to the challenge controls ($4{\cdot}5 \pm 0{\cdot}9$/VCU). No difference was found in intralamina propria lymphocytes in any of the groups examined; numbers varied from $15{\cdot}9 \pm 6{\cdot}3$/VCU to $20{\cdot}1 \pm 12{\cdot}8$/VCU. Peripheral EOS were not significantly

Table 2. Numbers of EOS and MMC per VCU in the small intestine of rats given various T-cell populations

Exp. no. (no./group)	T TDL	OX8$^-$	OX22$^+$	OX22$^-$	Challenge control
Exp. 1 (3)	51·4 ± 7·0*	66·3 ± 1·1	42·5 ± 2·5‡	59·9 ± 9·2	26·9 ± 7·6
	59·2 ± 15·8†	74·1 ± 43·6	94·2 ± 44·6	24·0 ± 7·7‡	17·8 ± 3·7
Exp. 2 (4)§	71·9 ± 8·7	—	29·5 ± 4·2‡	58·3 ± 13·3	29·5 ± 4·8
	10·7 ± 5·9		53·1 ± 12·0	11·5 ± 10·4	10·0 ± 6·8
Averages¶	62 / 35	66 / 74	31 / 71	55 / 15	27 / 12

* Numbers of EOS/VCU (above line).

† Numbers of MMC/VCU (below line).

§ There were four rats in each group except in the group which received T TDL cells. There were three rats in this group.

¶ The averages of numbers of EOS/MMC for T TDL or OX8$^-$ cell recipients were derived from Exps 1 and 2 or Exp. 1 only. For OX22$^+$, OX22$^-$ cell recipients and the challenge control group, the averages were calculated from five different experiments.

In Exp. 1, recipient rats were injected i.v. with 4×10^8 T TDL, 2×10^8 OX8$^-$, $1{\cdot}1 \times 10^8$ OX22$^+$ or $2{\cdot}1 \times 10^8$ OX22$^-$ cells, respectively. In Exp. 2, $3{\cdot}3 \times 10^8$ T TDL, $1{\cdot}1 \times 10^8$ OX22$^+$ or 2×10^8 OX22$^-$ cells were given, respectively. All rats were given a challenge infection of 1000 ML orally 24 hr after transfer of cells and the small intestines were examined histologically 9 days after challenge infection. Data represent means ± 1 SD of three to four rats per group.

elevated on Day 9 (P values all $>0{\cdot}05$) in rats transfused with isolated T-cell subsets (1227 ± 1093, 1413 ± 1404, 889 ± 339, and 557 ± 175/μl for rats receiving: TDL, OX8$^-$; OX22$^+$ and OX22$^-$ cells, respectively) compared to the challenge controls (703 ± 378/μl blood).

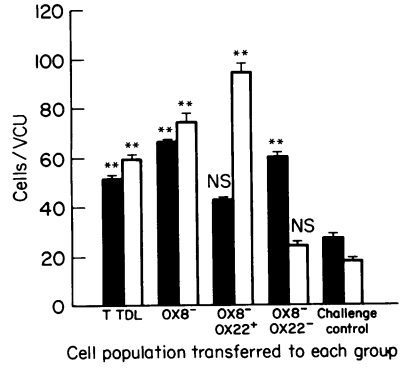

Figure 2. Intestinal eosinophil and MMC numbers after transfer of isolated T-helper subsets. Immune TDL cells were collected from rats 3 days after infection with 2000 ML. After isolation of T cells and T-helper subsets, four groups of recipient rats were injected i.v. as shown above (see text for details). Recipient and control rats were challenged with 1000 ML 24 hr later. Intestinal tissue was taken for histological processing 9 days after the challenge infection. A total of at least 10 VCU/section was counted and the results represent mean numbers of EOS (■) or MMC cells (□) VCU/rat ± SE of three rats per group. ** $P<0{\cdot}01$ compared with controls; NS, not significant.

Kinetics of intestinal eosinopoiesis and mastopoiesis after transfer with OX22$^+$/$^-$ T-helper subsets

The kinetics of increase in EOS and MMC were examined in an experiment in which four groups of rats (4/group) received 2×10^8 OX22$^-$ cells i.v. and another four groups of rats were given 2×10^8 OX22$^+$ cells i.v. These cell recipients and four groups of control rats (3/group) not given cells were then infected with 1000 ML 24 hr after cell transfer. On Days 3, 9, 15 and 21 after the challenge infection, the small intestine of rats receiving either OX22$^-$ or OX22$^+$ cells was removed and intestinal tissue at the centre of the first, second and third segments of the small intestine was taken and processed for histology. Control rats received neither cells nor an infection but were sampled as above on Day 0.

As shown in Fig. 4, normal rats had about 20 EOS/VCU through the whole length of the small intestine. When rats were infected with ML, the number of EOS/VCU increased significantly by Day 3 in the first 1/3 of the small intestine (34 ± 3). Peak eosinophilia in challenge control rats occurred on Day 15 for the first third (57 ± 3) and on Day 21 for the mid third (41 ± 1) of the small intestine. For the OX22$^+$ cell recipients, the kinetics of intestinal eosinophilia in the upper third of the small intestine were almost identical to those of the challenge control rats. There was, however, a significant increase of EOS/VCU in the lower two-thirds of the small intestine on Day 15. In recipients of OX22$^-$ cells, significant eosinophilia occurred by Day 3 and the number of EOS/VCU was nearly double that of the challenge control rats. On Day 9, EOS were still significantly higher than either the challenge control or the OX22$^+$ cell

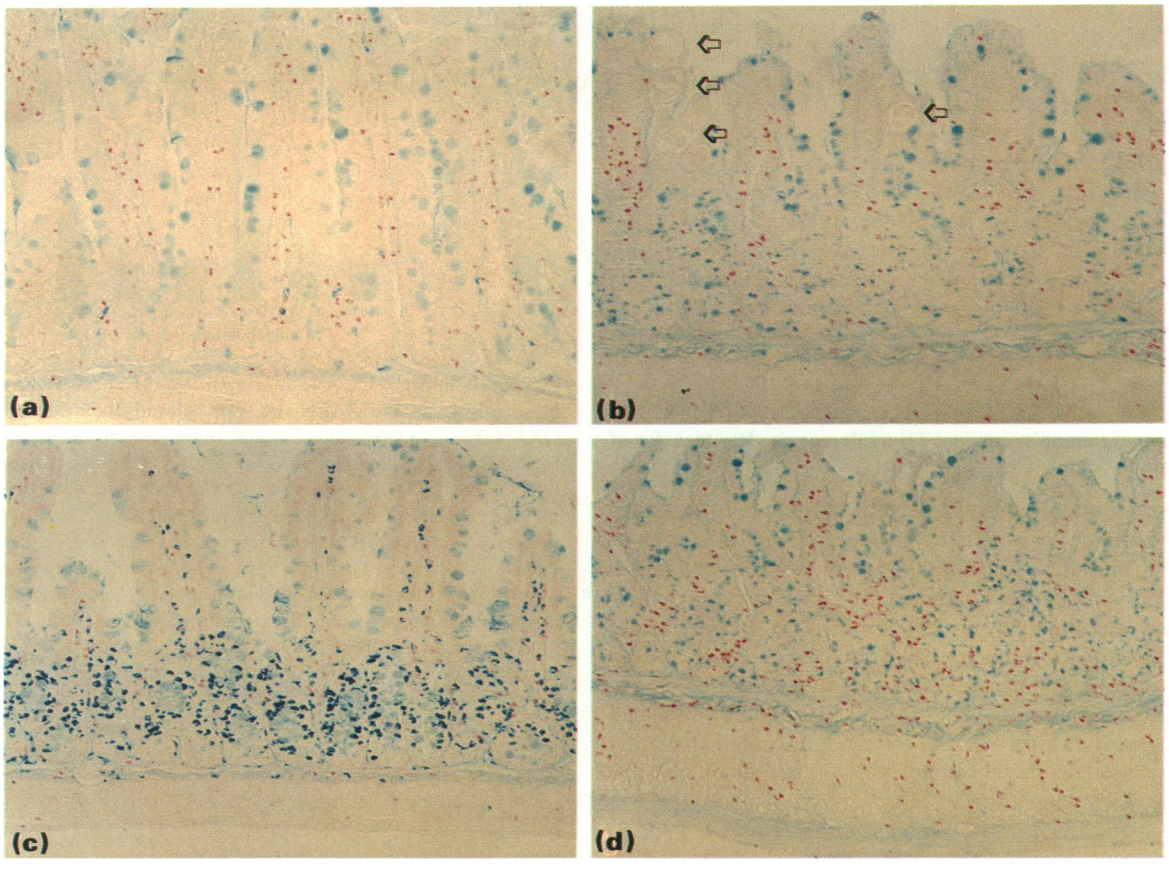

Figure 3. Tissue sections of the small intestine showing the increase in EOS and MMC 9 days after cell transfer. Intestinal tissue obtained from: (a) normal rats; (b) rats that had been infected with 1000 ML for 9 days; (c) recipients of $OX22^+$ T-helper cells (2×10^8/rat); (d) recipients of the $OX22^-$ T-helper cells (2×10^8/rat). Both groups of cell recipients were given a challenge infection with 1000 ML 24 hr after cell transfer and the intestinal tissue was taken from these rats on Day 9 after challenge infection. EOS (pink) and MMC (dark blue) were stained concomitantly with Alcian blue/Lendrum's solution (Magnification × 90). Arrows in (b) represent adult TS cut in cross-section.

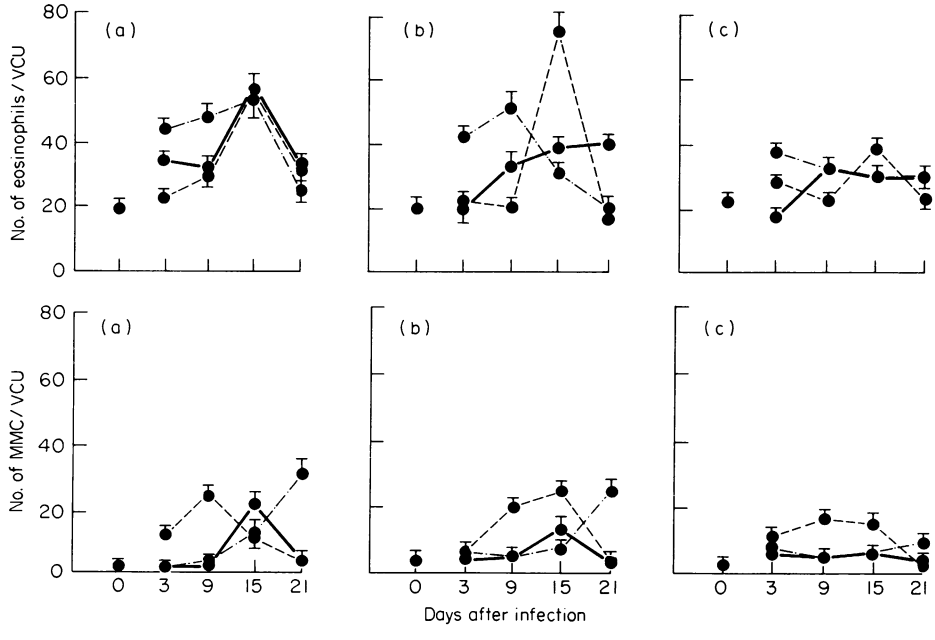

Figure 4. Kinetics of intestinal EOS and MMC after transfer of $OX22^{+/-}$ T-helper subsets. Rats were injected with 2×10^8 $OX22^+$ T-helper cells (– – – –) or 2×10^8 $OX22^-$ T-helper cells (–·–·–·–) i.v. on Day 0. The cell recipient rats and challenge controls (——) were infected with 1000 ML on Day 1 and the anterior (a), middle (b) and posterior (c) third of small intestine of these rats was examined histologically on Days 3, 9, 15 or 21. Normal non-infected non-transferred control rats were assayed on Day 0. Data represent mean numbers of EOS or MMC/VCU ± 1 SE for four (except the control group, which had three rats) per group.

recipients in the upper two-thirds of the small intestine. In normal rats, only a few MMC (3–4/VCU) were found. In challenge control rats, MMC/VCU increased to 22±2, 13±2, 7±1 in the three segments of the small intestine by Day 15 and then declined to normal levels by Day 21. In recipients of $OX22^+$ cells, mastocytosis was evident at Day 3 (12±1/VCU) and peaked on Day 9 for the anterior (25±1) and the posterior third (17±1) segments and on Day 15 for the middle third of the intestine (25±2). By Day 21, the number of MMC/VCU was normal again. In the $OX22^-$ cell recipients, mastocytosis did not appear until Day 21 in the upper two-thirds.

Expression of immunity against TS adult worms after transfer of Th subsets

To correlate intestinal cellular and morphological changes induced by the two subsets of T-helper cells with degree of protection, intestinal adult worms were also counted on Days 3, 9, 15 and 21 in the above experiment. As shown in Fig. 5, on Day 3 no worm rejection was evident but, by Day 9, a significant reduction in adult worm burden had occurred in the $OX22^-$ cell recipients alone compared to the challenge controls or the $OX22^+$ cell recipients. By Day 15 and Day 21, adult worms had been rejected from all rats. There was no difference in kinetics of worm rejection in the three portions of the small intestine examined (data not shown).

Effect of Th subsets on antibody-producing cells

The FIPA assay was used to determine which subset of Th cells stimulated specific B-cell proliferation. In this experiment, $OX22^+$ and $OX22^-$ Th subsets were injected i.v. into two groups of recipients (2×10^8 cells/rat). These rats and their controls were challenged with 1000 ML 24 hr later. Spleen and MLN cells were obtained 9 days after infection and the number of PFC was examined after incubation with TS adult antigen extract. The results (Fig. 6) showed that in challenge control rats there were 39·6±13·6 and 39·0±3·5 PFC/4 × 10^6 cells in spleen and MLN

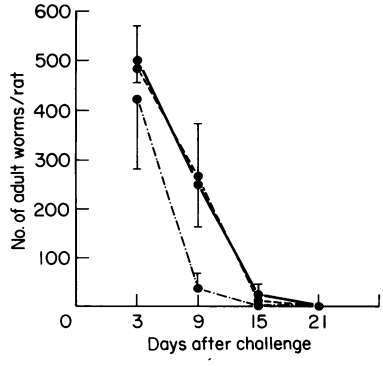

Figure 5. Expression of immunity against TS adult worms after adoptive transfer of T-helper subsets. Adult worm burden in the small intestine was assessed on Days 3, 9, 15 or 21. Data represent mean numbers of adult worms/small intestine±SE for four rats per group (the control group had three rats). Significant ($P<0·05$) reduction of adult burden occurred only on Day 9 in rats that had received the $OX22^-$ T-helper cells (–·–·–·–). No difference ($P<0·05$) was found between the $OX22^+$ cell recipients (– – – –) and the challenge controls (——).

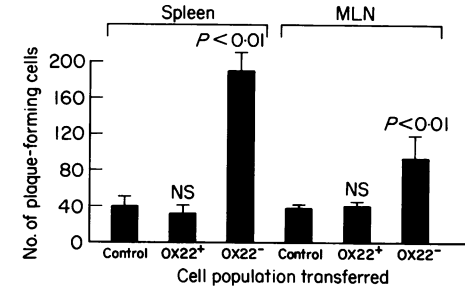

Figure 6. Number of specific anti-*T. spiralis* adult antibody-forming cells in the spleen and MLN of rats adoptively transferred with $OX8^-OX22^+$ or $OX8^-OX22^-$ cells. Immune TDL cells were collected from donor rats 3 days after infection with 2000 muscle larvae. T-helper cells of $OX8^-OX22^+$ and $OX8^-OX22^-$ subsets were isolated and injected i.v. into two groups of recipient rats (2×10^8 cells/rat), respectively. These rats and their normal controls were challenged with 1000 muscle larvae 24 hr later. Spleen and mesenteric lymph node cells were obtained 9 days after challenge infection and antibody plaque-forming cells assessed using the filter immuno-plaque assay. The specific antigen used was TS adult extract at 25 μg/ml, 0·25 ml/well. The primary antibody used was rabbit anti-rat Ig $F(ab')_2$ (1:100 dilution) and peroxidase-conjugated goat anti-rabbit IgG (H- and L-specific, 1:100 dilution). Data represent mean (±1 SD) numbers of PFC/4 × 10^6 cells obtained from groups of rats containing four rats per group. NS, not significant.

lymphocytes. There was no increase in PFC in spleen or MLN in recipients of $OX22^+$ cells. In the $OX22^-$ cell recipients, however, both the spleen (190·1±20·9) and the MLN (94·1±25·4) had significantly higher numbers of PFC. Again, it was the $OX22^-$ cell recipients that had reduced the adult worm burden (156±61, $P<0·01$) compared to the challenge controls (358±68). The intestinal worm counts of the $OX22^+$ cell recipients were not significantly decreased (266±86, $P>0·05$). Similar changes in EOS and MMC were also evident in the small intestine of these rats, e.g the $OX22^+$ cell recipients had increased MMC (66±19/VCU, $P<0·01$) and EOS (34±11/VCU, $P<0·05$) whereas the $OX22^-$ cell recipients had increased EOS (46±10/VCU, $P<0·01$; MMC/VCU was 15±11, NS) compared to the controls (14±3 EOS/VCU, 13±5 MMC/VCU). The goblet cells/VCU in the $OX22^+$ (44±16) or OX22- cell recipients (38±14) did not differ significantly from their challenge controls (31±10).

DISCUSSION

The results reported here demonstrate that distinct subsets of thoracic duct T cells obtained from TS-infected rats promote eosinophilia and mucosal mastocytosis in the small intestine of naive recipient rats. With a challenge infection, eosinophilia and mastocytosis are further enhanced, suggesting that the infection provides an additional stimulus leading to an amplification of the underlying intestinal cellular changes initiated by transferred cells. However, the most important finding of this study is that the induction of intestinal mastocytosis is a function of the $OX8^-$ $OX22^+$ Th subset and that eosinophilia in the intestine, worm rejection and antibody formation are all mediated by the $OX8^-$ $OX22^-$ Th subset. These results demonstrate the func-

tional uniqueness of the Th subsets defined by OX22 mAb and further indicate that MMC may not be required for worm expulsion during the primary infection, as suggested by Parmentier *et al.* (1987).

During an uninterrupted TS infection, eosinophilia and mastocytosis reached their peak on Day 15 in the anterior two-thirds of the small intestine (Fig. 4). This is the preferred intestinal habitat of adult TS and the site at which immunity is first manifest. The kinetics of mastocytosis and eosinophilia described here are essentially identical to those reported from several laboratories with different intestinal helminth infections in rodents (Alizadeh & Murrell, 1984; Ruitenberg *et al.*, 1977; Tronchin *et al.*, 1979; Mayrhofer & Fisher, 1979; Woodbury *et al.*, 1984; Wakelin & Donachie, 1983). The linkage of worm rejection in the primary infection with increased numbers of intestinal EOS and/or mast cells has been a theme of numerous authors (Rothwell, 1975; Woodbury *et al.*, 1984). However, there has never been a direct test of the association of any of these inflammatory changes with the process of rejection. Earlier experiments from this laboratory had shown that positively selected T-helper cells were essential for transferring protection and that the activated $OX22^-$ subset was the effector population (Korenaga *et al.*, 1989). The experiments reported here were designed to explore the *in vivo* properties of these cell subsets in more detail. Complex interactions were suggested by the fact that distinct temporal patterns of MMC and EOS waxing and waning were evident with both transferred cell populations. These changes were superimposed on a fully reactive TS-infected cell recipient. Therefore, we believe the early changes (up to Day 9) more clearly and fully reflect the functional activity of the transferred cells (Figs 2 and 4). Day 9 signals the initiation of increased mastocytosis and eosinocytosis in challenge controls. Of the two effects, the increase in MMC was the most striking and clearly a property of $OX22^+$ cells. Increases in eosinophils were observed with both cell populations but were most pronounced with the $OX22^-$ cells. The weak degree of eosinophilia observed with $OX22^+$ cells (Table 1, Exp. 1) was largely explained by the degree of contamination of this cell population with $OX22^-$ cells (Fig. 1). The reasons for this contamination: the low expression of OX22 on $CD4^+$ cells, have been addressed before (Korenaga *et al.*, 1989).

Adoptive transfer of the $OX22^-$ Th cells accelerates peak eosinophilia in the small intestine by 6 days, and transfer of $OX22^+$ Th cells advanced intestinal mastocytosis by the same period (Fig. 4). However, only the $OX22^-$ Th subset was able to expedite worm rejection from the gut (Fig. 5), as demonstrated previously (Korenaga *et al.*, 1989) and as shown in at least 10 other unreported experiments in this laboratory. These experiments indicate that MMC are, at the least, not necessary nor sufficient by themselves for the worm rejection episode that terminates the primary infection. These experiments and the data of Parmentier *et al.* (1987) are the first to break the temporal association between mast cell hyperplasia and parasite rejection and this is an essential step if we are to move beyond correlations to mechanisms. Peak eosinophilia coincides with the time of adult worm rejection in the recipients of $OX22^-$ T-helper cells. However, this too provides no more than a temporal association which may relate more to the stimulatory properties of the infection on transfected cells and the specific cytokines produced by these cells than to any role of EOS in the rejection process. The earlier work of Dessein *et al.* (1981) also suggested a possible role for EOS in protection. In addition, protective $OX22^-$ cells also stimulated a large increase in B cells making anti-adult TS antibodies, and a role for antibodies in parasite expulsion has not yet been experimentally excluded. Experiments aimed at defining the role of antibody in the primary rejection process have not been conclusive as not only was a role for B cells suggested by some experiments in mice (Wakelin & Wilson, 1979), but the possibility that transferred T cells may stimulate B-cell differentiation, as shown here, was not considered. The fact that transfer of $OX22^-$ Th cells significantly increases the number of specific antibody-producing B cells (Fig. 5) confirms earlier findings (Spickett *et al.*, 1983) that only the $OX22^-$ but not the $OX22^+$ Th subset provides help for B cells in specific antibody responses. In rats, B cells have been shown to transfer some anti-TS protection by themselves and the protective effect of non-dividing T cells has been ascribed to the provision of help for B cells (Crum, Despommier & McGregor, 1977). However, there is no direct proof as yet that either inflammation and/or antibody formation are essential components of the primary worm expulsion process for TS.

Peripheral EOS were not significantly elevated after transfer of $OX22^-$ cells, suggesting that the effects of the $OX22^-$ T cells were predominantly local and may be confined to the gut. The anatomically restricted patterns of intraintestinal distribution of EOS and MMC (Fig. 3) also suggest that the two subsets of T cells may exert their effects quite locally and at different sites, perhaps through selective intraintestinal migration. Our experimental analysis has shown that while both $OX22^+$ and $OX22^-$ T cells migrate in large numbers to the small intestine, their migratory behaviour within the gut is different (Wang *et al.*, 1990). In mice it has been shown that TS infections initiate a local expansion of mast cell precursors present in the gut (Dillon & McDonald, 1986) and our observations here are consistent with this view for both the MMC and EOS.

It has been suggested that all activated T cells lose LCA as a part of the activation process (Akbar *et al.*, 1988). If this were the case then we could expect that there would be no functional differentiation between the $OX22^+$ and $OX22^-$ cell populations since, as $OX22^+$ cells became activated, they would lose $OX22^+$ expression. There is no doubt that protection is a property of the dividing cells in the $OX22^-$ cell subset (Bell *et al.*, 1987). The $OX22^+$ cell population has failed to transfer protection in 15 distinct experiments utilizing varying cell doses, as we have reported previously (Korenaga *et al.*, 1989). However, there are also dividing cells in the $OX22^+$ subset and these cannot be entirely accounted for by contamination ($\sim 10\%$) with $OX22^-$ cells. Although we have not directly shown that mastocytosis or eosinopoiesis are stimulated by the dividing cells in each subset, these seem to be the most likely candidates. This suggestion also corresponds with the view that the high MW form of LCA, i.e. the target of $OX22^-$ mAb, is a differentiation marker lost as cells become activated. However, results for eosinopoiesis/mastopoiesis, antibody help, intraintestinal cell migration as well as worm rejection, are all consistent with the view that the presence or absence of OX22 on Day-3 TDL from infected rats corresponds with distinct functional properties of these populations. These data both corroborate and extend those of Spickett *et al.* (1983). We cannot determine whether there is a functional parallel between the $OX22^+$ and $OX22^-$ cell subsets and Th1 and Th2 as defined in the mouse, but this has been suggested based on the presence of OX22 (Akbar *et al.*, 1988; Bottomly,

1988). One immediate question is that in our hands only OX22$^+$ cells induce MMC hyperplasia whereas in mice both Th1 and Th2 are known producers of IL-3, and Th2 produce an additional mast cell growth factor (Mosmann *et al.*, 1986; Woods *et al.*, 1987), suggesting that Th1 and Th2 may not be easily differentiated on the basis of mastopoiesis alone. Our data suggest a more precise allocation of mast cell-stimulating activity between OX22$^+$ and $^-$ cell subsets. This question can only be resolved by more detailed *in vitro* analysis of the cytokines produced by activated OX22$^+$ and OX22$^-$ cells, as well as by more detailed analysis of the Ig isotypes induced by transfer of OX22$^-$ cells. We failed to find any increase in goblet cells/VCU after transfer of T-helper cells of either phenotype, although this has been reported previously (Miller & Nawa, 1979; Ahlstedt & Enander, 1987) using whole thoracic duct T-cell populations.

In summary, our results have demonstrated a clear difference between the OX22$^+$ and OX22$^-$ T-helper subsets in their capacity to stimulate MMC and EOS proliferation, respectively, in the small intestine at an early stage of TS infection. Only OX22$^-$ cells are able to activate specific antibody-producing B cells in the MLN and spleen of recipient rats and to stimulate rejection of TS adult worms from the small intestine. The results suggest that intestinal mast cell proliferation is not required for parasite rejection to occur in the primary infection. In addition, the functional dichotomy between these subjects is consistent with the view that OX22 expression is a property of functionally distinct T-helper subsets in the rat.

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