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Intestinal immunity to *Trichinella spiralis* is a property of OX8$^-$ OX22$^-$ T-helper cells that are generated in the intestine

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SUMMARY

The phenotype of T-helper cells conferring protection against *Trichinella spiralis* (Ts) was studied using adoptive transfer procedures and T-helper cell subsets isolated by monoclonal antibodies. With these techniques OX8$^-$ OX22$^+$ and OX8$^-$ OX22$^-$ T-helper cell populations were isolated from thoracic duct lymph (TDL) of infected rats three–five-fold more concentrated than in unfractionated lymph. The OX8$^-$ OX22$^-$ cell subset alone transferred enhanced rejection of adult worms from the intestine. The origin of protective OX8$^-$ OX22$^-$ cells was examined in mesenteric lymphadenectomized (MX) rats. After MX, protective cells were found in the cell population draining directly from the intestine on Days 2–3 after infection. Protective cells first appeared in the mesenteric lymph node (MLN) and efferent lymph at Day 3. MX rats rejected *T. spiralis* at the same time as intact controls and showed enhanced rejection when immune TDL were transfused. No evidence was found for a direct role of the MLN in the generation or expression of parasite rejection. Depletion of migrating OX8$^-$ OX22$^-$ blast cells by 48-hr drainage of TDL did not influence the expression of an anamnestic response to challenge infection. This suggests that an intestinally resident cell population has a substantial role in mediating primary worm rejection and anamnestic immunity. Day 2 OX8$^-$ OX22$^-$ cells from MX rats proliferated in response to the presentation of adult and muscle larvae antigens *in vitro*. We conclude that protection resides in the OX8$^-$ OX22$^-$ T-helper cell subset that is produced and functions in the intestine.

INTRODUCTION

In rodents, the capacity to reject adult *Trichinella spiralis* (Ts) from the intestine has been shown to be a transferable T-cell function. In mice, protective T helper cells (L3T4$^+$) have been demonstrated in the mesenteric lymph node (MLN) from Days 4–8 (Grencis & Wakelin, 1982; Grencis, Reidlinger & Wakelin, 1985) after the primary infection, whereas in rats protective T-helper cells are present in thoracic duct (TD) lymph from Days 3–4 for a total period of about 48 hr (Bell, Korenaga & Wang, 1987). The evanescent appearance of protective T-helper cells appears to be a characteristic feature of the immune response to Ts in both rodent species, suggesting that the migratory effectors of rejection are short-lived. In neither host species has a site of origin of protective cells or the site of action of T-helper cells been determined. These are important steps in defining mechanisms leading to rejection of intestinal helminths, as the site of action and type of intestinal effector may be determined by the origin and means of stimulation of the initial reactive cell population. The early dynamics of cell subpopulation interactions have been shown to influence resistance/susceptibility to *Schistosoma mansoni* in rats (Phillips *et al.*, 1987).

The phenotype of reactive cells is also a major influence on the type of response that is ultimately produced. Specialization in lymphokine production among T-helper (Th) cell subsets restricts and directs the responses that can be promoted in target cell populations. Thus, in mice, Th1 produce IL-2 and interferon-gamma, leading preferentially to delayed hypersensitivity, secretion of IgG2a and inhibition of IgE and IgG1 production, whereas Th2 cells stimulate IgE and IgG1 production through secretion of IL-3 and IL-4 (Mosmann *et al.*, 1986; Finkelman *et al.*, 1986, 1988). The direction of this reciprocal interaction between T-cell subsets is likely to be determined by the type and specificity of the initial stimulus to divide and differentiate received by the responsive T-cell population. Intestinal helminth infections, including Ts, have long been recognized to promote high levels of IgE and IgG1 (Dessein *et al.*, 1981), thus

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Abbreviations: GaMG, goat anti-mouse IgG; MA, metabolic antigen; ML, muscle larvae; MLN, mesenteric lymph node; MX, mesenteric lymphadenectomy; R/I, regional/intestinal; sIg, surface immunoglobulin; TD, thoracic duct; Ts, *Trichinella spiralis*.

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suggesting the selective activation of T-helper cell subsets presumably corresponding to Th2. In rats, T-helper cells (W3-25 = CD4 and Ly 1) can be subdivided based on the presence or absence of a cell surface marker identified by monoclonal antibody OX22 directed against the leucocyte common antigen (Woollett *et al.*, 1985). The $OX22^-$ T helper provides help for antibody-forming B cells, whereas the $OX22^+$ T helper provides help for cytotoxic/suppressor T cells (Mason *et al.*, 1983; Spickett *et al.*, 1983). Using the MRC OX22 monoclonal antibody, we have examined the capacity of both subsets of T-helper cells to transfer immunity adoptively against Ts.

MATERIALS AND METHODS

Animals

Male and female AO strain rats aged 6–10 weeks were used throughout these experiments. All rats were bred at the Baker Institute vivarium where they were maintained on a 12-hr light/dark cycle and given food and water *ad libitum*.

Trichinella spiralis

Procedures for maintaining the parasite, isolating larvae, infecting rats and counting adult worms have been described previously (Bell, McGregor & Adams, 1982). In one experiment, the production of newborn larvae was suppressed by feeding rats a diet containing 0·5% thiabendazole (kindly donated by Merck & Co., Rahway, NJ) which prevents the establishment of muscle larvae (ML) (Bell, McGregor & Despommier, 1979). Cell recipients were infected 1 day after cell transfer, except where indicated with the recipients of TDL from mesenteric lymphadenectomy (MX) rats, and worm counts were always performed 9 days after infection (Bell *et al.*, 1987).

Parasitic antigens

Two types of antigen were used; whole parasite antigens were prepared by sonicating large numbers of worms (ML or adults) at high frequency in a Branson Sonifer (Plainview, NY). Metabolic antigens (MA) were prepared *in vitro* from adult and larval worms as previously described (Bell *et al.*, 1983).

Antibody

OX8 and OX22 monoclonal antibodies were either purchased from Accurate Biochemical Corp. (Westbury, NY) or obtained from ascitic fluid produced at the Baker Institute from OX8 and OX22 hybridoma cell lines (a gift from Dr A. Like, University of Massachusetts) with permission from Dr A. Williams (Oxford University, U.K.). Fluoresceinated GaMG and goat anti-rat IgG were obtained from Cappel Laboratories (Malvern, PA). Polyclonal sheep and rabbit anti-rat $F(ab')_2$ were produced at the Baker Institute.

Surgery

The thoracic duct (TD) was cannulated according to the basic method of Bollman, Cain & Grindlay (1948). The procedure was identical for both normal and MX rats. The method of Steer (1981) was used to produce MX rats. Briefly, the peritoneal cavity of anaesthetized weanling rats was opened on the midline and the intestines reflected laterally to expose the caecum. The large and smaller single nodes were removed by blunt dissection using forceps and cotton-tipped swab-sticks. Treated rats were left to recover for a minimum of 4 weeks to allow lymphatic regeneration. After this they were used in experiments along with age-matched controls. Throughout this paper we refer to the lymph collected from MX rats as regional/intestinal (R/I) lymph. Strictly speaking, this lymph is not truly afferent as the TD collects efferent lymph from the posterior abdomen and hindquarters. However, afferent lymph from the gut makes the largest single contribution to the cell population carried. Identical surgical procedures were used to obtain MLN cells for use in adoptive transfer experiments.

Cells

Cannulation was routinely done 3 days after infection (except where stated otherwise) and TD lymph was collected at room temperature for 16–24 hr into heparinized Ringer solution without antibiotics. Cells were washed twice in Hanks' balanced salt solution (HBSS) + 5% fetal bovine serum (FBS) at 400 *g* for 10 min before adjustment to 2×10^7/ml in HBSS for affinity chromatography or other separation procedures. In some experiments twice-washed unfractionated TD lymphocytes were infused without further manipulation (see text).

Affinity chromatography of cells

This procedure was undertaken as described elsewhere (Crum & McGregor, 1976), to remove B cells from the TD lymph cell population prior to separation of T cells by negative selection. The separated T-cell population (sIg^-) was 95–98% definable using T-cell markers.

Separation of cell subsets

Recovered T cells were further fractionated using monoclonal antibody OX8, specific for rat cytotoxic/suppressor cells (Mason *et al.*, 1983), or OX22, which splits T-helper cells into those providing help for T-cytotoxic/suppressor cells ($OX22^+$) or those providing help for B cells ($OX22^-$) (Spickett *et al.*, 1983). The panning method described by Wysocki & Sato (1978) was used to separate each cell subset. Purified T cells obtained from affinity columns were incubated for 20 min at 4° in RPMI-1640 containing a 1 : 100 dilution of OX8 antibody. After two washes T cells were incubated for 30 min at room temperature in petri-dishes coated with affinity-purified goat anti-mouse IgG (GaMG). The latter comprised a mixture of 10–25% affinity-purified GaMG in normal goat IgG. Non-adherent cells were poured off after gentle rotation of the petri-dish for 30–45 seconds to resuspend non-adherent cells. Adherent cells were then removed by vigorous pipetting. Cells were recovered with greater than 99% viability. The purity of each subset was checked by incubating separated cells with mAb OX8 or OX22 followed by an affinity-purified fluoresceinated anti-mouse IgG (H and L chain) antibody. Cells were examined for fluorescence on a FACS IV (Becton-Dickinson, Sunnydale, CA). The $OX8^-$ subset was routinely 90–95% pure. The $OX8^-$ $OX22^+$ and $OX8^-$ $OX22^-$ subsets were $\geq 80\%$ pure. Contamination of the $OX22^-$ subset with $OX22^+$ cells ($\sim 10\%$) occurred due to the weak expression of OX22 by $OX8^-$ T-helper cells (Spickett *et al.*, 1983). In addition, negative selection concentrates cells that do not express any known T- or B-cell surface markers. These cells comprise 1–3% of the initial population, but as much as 4–9% of final negatively selected cell subsets.

Table 1. Adoptive transfer of immunity with efferent TD T-helper cell subsets

Exp.	Cell population transferred	No. of recipients	Worm burden	Probability
1	$2{\cdot}1 \times 10^8$ OX8$^-$ OX22$^-$	4	167 ± 35	$<0{\cdot}001$
	$1{\cdot}1 \times 10^8$ OX8$^-$ OX22$^+$	4	385 ± 29	NS
	No cell control	4	302 ± 53	
2	1×10^8 OX8$^-$ OX22$^-$	6	156 ± 61	$<0{\cdot}001$
	1×10^8 OX8$^-$ OX22$^+$	4	266 ± 86	NS
	No cell control	5	358 ± 68	

In experiment 1, cells were transferred in the approximate proportions recovered after separation. In the second experiment, cell numbers transferred were the same for each subset. Cell recipients were infected with 1000 Ts the day after cell transfer and worm burden was assessed at 9 days. Results are expressed as the mean worm burden $\pm$ 1 SD for each group.

Blastogenic cell assay

[Methyl-^{3}H]thymidine (Amersham Corp., Arlington Hts, IL) uptake was assessed directly on fractionated cell populations derived from TD lymph to determine the subset distribution of dividing cells at the time of collection. Freshly collected affinity-purified TD T lymphocytes were fractionated by panning as outlined above. Each subset was washed once in RPMI-1640 and 2×10^7 cells were incubated in 10 ml of RPMI containing 10% FBS and 2 μCi [^{3}H]thymidine per ml. Cells were incubated for 2 hr at 37° in a water-bath with occasional gentle stirring. Labelled cells were washed three times in PBS + 1% FBS before counting in a Beckman liquid scintillation counter.

In vitro *proliferation assay*

Defined subsets of TD T lymphocytes were washed twice with RPMI-1640 containing 2% normal rat serum and resuspended in RPMI-1640 containing 5% rat serum. Then, 0·1 ml of each subset ($2{\cdot}5 \times 10^6$/ml) and 0·1 ml antigen-presenting cells (2×10^5/ml) containing *T. spiralis* antigens were added to each well of a 96-well round-bottomed microtitre plate. Forty-eight hours after initiation of the culture, cells were pulsed with 0·5 μCi-[^{3}H]thymidine and cultured for a further 24 hr before harvesting to estimate [^{3}H]thymidine uptake.

Preparation of antigen-presenting cells

The spleen was removed from an AO rat and a cell suspension made by rubbing the spleen through a stainless steel sieve. Erythrocytes were lysed with 4 ml of Tris-buffered ammonium chloride for 2 min on ice. Thereafter the cells were washed twice. Cell suspensions (2×10^5/ml) were made in RPMI containing 5% rat serum. Different antigens were added to the cell suspensions (10 μg/ml for MA, 100 μg/ml for crude antigens) respectively. Cells were incubated in a CO_2 incubator and then irradiated with 1000 rads. The cells were then washed twice and resuspended in RPMI containing 5% normal rat serum with an equivalent volume of added antigens.

Statistics

The significance of differences in mean values between two groups was examined by Student's *t*-test. *P* values $<0{\cdot}05$ were considered significant.

RESULTS

Further characterization of protective cells in efferent TD lymph

Previous experiments (Bell *et al.*, 1987) have shown that highly purified, positively selected W3-25$^+$ cells are protective in adoptive transfer systems, and the following experiments were designed to localize further protective capacities within the W3-25$^+$ population using the OX22 mAb. In the first experiment (Table 1) cells were transferred in the approximate proportions that they had been recovered after panning, whereas in the second experiment equal numbers of cells of both phenotypes were transferred. In both cases and in all subsequent experiments protection was found only in the OX22$^-$ helper cell subset.

The presence of protective cells in the mesenteric node

MLN cells were harvested at Days 2–5 from infected rats and adoptively transferred by intravenous injection to naive rats. Cell recipients were challenged with 1000 ML the day after cell transfer, as done for adoptive transfer of TD lymphocytes. Assessment of the protective value of MLN cells showed that only those collected on Days 3 and 4 could transfer protection (Fig. 1). The presence of protective cells in the MLN at precisely the same time as they are found naturally migrating in efferent TD lymph (Bell *et al.*, 1987) suggests that protective cells might be produced in the MLN.

The role of the mesenteric node in protection

Initially, we determined the capacity of MX rats to reject a Ts infection. To do this, MX and sham-MX rats were infected with muscle larvae 6 weeks after surgery. The worm burden was assessed during late rejection when any quantitative deficiency of MX rats should have been most pronounced. There was no difference in worm burden 12 days after infection (MX,

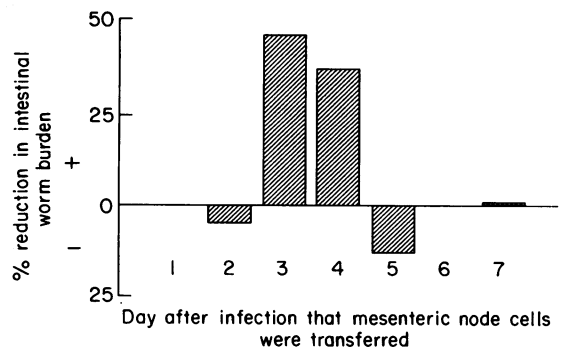

Figure 1. Percentage rejection of Ts after transfer of MLN cells collected on the designated days. Only cells collected on Days 3 and 4 were capable of transferring protection. A negative value indicates that worm burden in cell recipients was higher than in controls. None was significantly higher.

Table 2. Protective properties of adoptively transferred R/I lymph cells demonstrated after a 48-hr delay in challenge

Group	Cell population transferred	Time after cell transfer of recipient infection	Worm burden	Probability
1	3×10^8 R/I cells collected on Day 3 injected i.v.	2 days	118 ± 24	$<0{\cdot}025$
2	3×10^8 R/I cells collected on Day 3 injected into MLN	2 days	113 ± 3	$<0{\cdot}0005$
3	No cells	2 days	190 ± 11	

136 ± 127; Sham-MX, 95 ± 31), when about 90% of the infecting dose had been rejected by both groups. In an extension of this experiment, we also showed that worm rejection occurred as effectively in MX rats given unfractionated protective Day 3 TD cells as in normal recipients (MX + cells, 28 ± 41; normal + cells, 67 ± 67; MX control, 407 ± 104; normal control 309 ± 47). Close examination of the viscera of these rats did not reveal regenerative lymphoid tissue in the area drained by intestinal lymphatics. These results suggest that protective cells in the MLN or TD lymph at Days 3 and 4 had originated elsewhere.

The presence of protective cells in R/I lymph

In order to focus on the protective ability of the cells that drained directly from the gut, MX rats were infected 6 weeks after surgery and TD cells were collected at daily intervals thereafter for adoptive transfer. Initially, we collected R/I cells from TDL donors on Days 1, 2 and 3 after infection and tested their protective capacity after infecting cell recipients 1 day later. Although this procedure is effective with efferent TD lymphocytes or MLN cells, protection was never found (data not shown). However, when Day 3 R/I cells were transferred to normal rats and these rats were challenged 2 days later, protection was observed (Table 2). In this experiment we also examined the possibility that residence in, or passage through, the mesenteric node was required by injecting R/I cells directly into the node. Although rats receiving cells in the node rejected worms faster than normal controls there was no difference between them and rats whose transferred cells were injected i.v.

To determine which subset of R/I lymphocytes at Day 3 was protective, T-helper cells (depleted by affinity chromatography of B cells and by panning of $OX8^+$ cytotoxic/suppressor T cells) were separated by panning using mAb OX22. Adoptive transfer of these two populations showed that it was the $OX22^-$ cells that conferred protection (Table 3). In an identical separation, $OX8^- OX22^-$ R/I lymph cells collected on Day 2 were also shown to carry protective functions (2×10^8 $OX8^- OX22^-$ cells/recipient, 54 ± 65 worms; no cell control, 529 ± 98, $P < 0{\cdot}0001$).

Depletion of migrating $OX8^- OX22^-$ cells

The contribution that migrating TD blast cells made to anti-Ts immunity in infected rats was assessed in the following experiment. Cell donors were cannulated and the fistulae allowed to run freely for 48 hr from Day 3 to Day 4. This procedure depletes greater than 90% of the migratory blast cells (Bell *et al.*, 1987). Controls consisted of rats whose fistulae had clotted shortly after surgery and had therefore not been depleted of migratory cells. The cannulae were removed after 48 hr and rats were fed 0·5% thiabendazole in the diet for 14 days. This procedure prevents ML establishment and the development of long-term rapid expulsion. When such rats are rechallenged they show an anamnestic anti-adult worm response leading to the rejection of adult worms by 5–6 days after infection (Bell *et al.*, 1979). Eight weeks after cannulation, depleted, non-depleted and normal rats were infected with 1000 ML and killed 6 days later for worm counts. The results showed that worm rejection was identical in depleted (1 ± 1 adults) and non-depleted rats (3 ± 4 adults; control, 417 ± 82).

Table 3. Adoptive transfer of $OX22^-$ and $OX22^+$ cells from R/I lymph to naive recipients

Group	Cell population transferred*	Challenge with†	Adults/SI Day 12‡
A	$OX8^- OX22^-$	1000 ML	194 ± 67
B	$OX8^- OX22^+$	1000 ML	586 ± 112
C	—	1000 ML	670 ± 34

* Cells were collected from rats cannulated 3 days after infection.
† Challenge with ML took place 2 days after cell transfer.
‡ $P < 0{\cdot}005$.

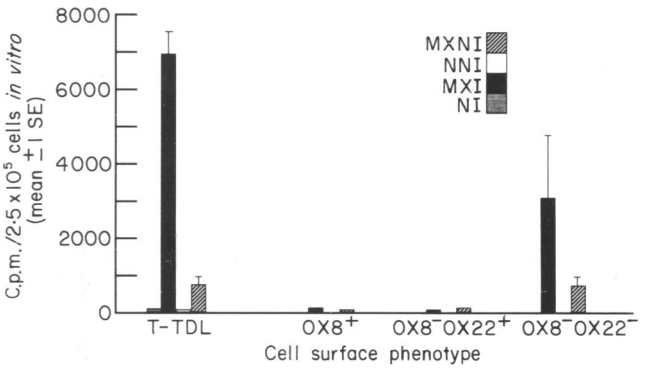

Figure 2. T-cell subset distribution of dividing cells in R/I lymph on Day 2–3 after infection. Essentially all dividing cells were present in the $OX8^- OX22^-$ T-helper cell subset of MX rats. No dividing cells were present in efferent TDL at this time. NI, not infected; I, infected; MX, mesenteric lymphadenectomy; N, normal.

Characteristics of R/I lymphocytes from infected rats

The kinetics of appearance of dividing cells in R/I lymph was monitored using short-term [^{3}H]thymidine uptake after subjecting the cells to the fractionation procedures outlined above. On Day 2 after infection, essentially all the dividing R/I cells belonged to the $OX8^- OX22^-$ T-helper cell subset (Fig. 2). There were no dividing cells present in efferent thoracic duct

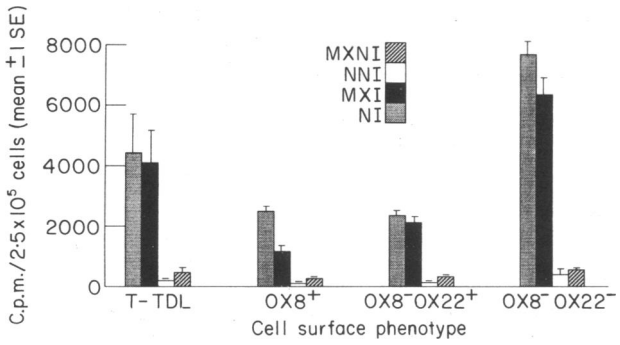

Figure 3. T-cell subset distribution of dividing cells in R/I lymph on Days 3–4 after infection. By this stage dividing cells are present in all T-cell subsets and in identical proportions in R/I lymph and efferent lymph. NI, not infected; I, infected; MX, mesenteric lymphadenectomy; N, normal.

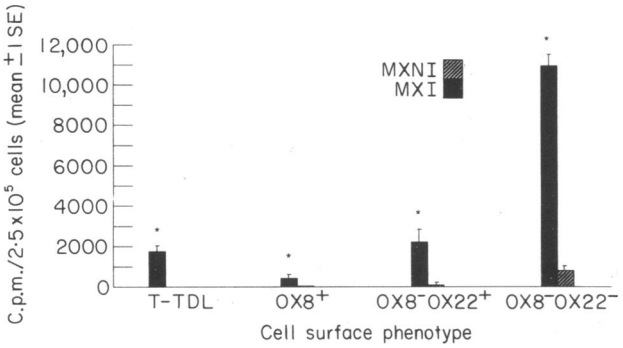

Figure 4. *In vitro* cell proliferation in response to stimulation with muscle larvae crude antigen. Cells collected from R/I lymph on Day 2. * Significantly different from the control. NI, not infected; I, infected; MX, mesenteric lymphadenectomy; N, normal.

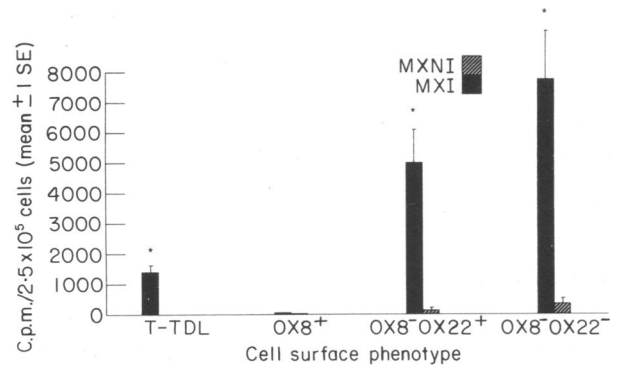

Figure 5. *In vitro* cell proliferation in response to stimulation with adult worm crude antigen. Cells collected from R/I lymph on Day 2. * Significantly different from the control. NI, not infected; I, infected; MX, mesenteric lymphadenectomy; N, normal.

lymph at Day 2 and these first appeared on Day 3 after infection (Bell *et al.*, 1987). By Day 3 dividing cells were present in all T-cell subsets of R/I lymphocytes including OX8$^+$ cytotoxic/suppressor cells and OX8$^-$ OX22$^+$ T helper cells (Fig. 3).

To determine whether the cells present in R/I lymph 2 days after infection were responsive to Ts antigens, Day 2 cells were cultured in the presence of crude antigen extracts from either adult or larval worms. OX8$^-$ OX22$^-$ cells responded positively to stimulation with both types of antigen (Figs 4 and 5). Interestingly, T-helper cells of the OX8$^-$ OX22$^+$ subset also showed a significant lympho-proliferative response, even though there were no dividing cells in this subpopulation when collected from the thoracic duct.

DISCUSSION

The experiments reported here extend earlier analyses of the T-cell population that confers protection against Ts in rats (Bell *et al.*, 1987). In particular, we have demonstrated that protection apparently resides solely in a subpopulation of T-helper cells of the phenotype OX8$^-$ OX22$^-$. These cells are generated in the intestine and dividing cells migrate into draining lymph beginning 2 days after the initial contact with antigen. At 2 days dividing cells (of OX8$^-$ OX22$^-$ phenotype) have specificity for antigens expressed by muscle larvae and adult worms. The infection also produces a local population of cells that does not migrate from the gut and can mount an anamnestic anti-Ts response. We additionally demonstrate that the MLN is not essential for the induction of protective responses or for effector function after adoptive transfer of protective cells.

Our isolation procedures concentrated OX8$^-$ OX22$^-$ cells by a factor of three–five-fold and protection could be demonstrated with less than half (1×10^8) of the calculated Day 3 output of these cells in efferent lymph (~ 2–3×10^8). In contrast, more than 1×10^8 OX8$^-$ OX22$^+$ cells failed to transfer any protection whatsoever. This represents more than 10 times the total contamination of 10% OX8$^-$ OX22$^+$ cells in the OX8$^-$ OX22$^-$ cell subset after panning. In addition, 10^8 OX8$^-$ OX22$^+$ cells approximates a full 24-hr output of these cells in infected rats. The OX8$^-$ OX22$^+$ cells were fully viable as measured by trypan blue exclusion and responsive *in vitro* to adult and muscle larvae antigens (Fig. 5 and data not shown). The failure of OX8$^-$ OX22$^+$ cells to transfer protection was therefore not due to an inability to recognize antigen. Furthermore, the only dividing cells in R/I lymph at 2 days are OX8$^-$ OX22$^-$ and these cells transfer protection. The evidence thus points strongly to the mediator of rejection in rats being the OX8$^-$ OX22$^-$ T-helper subset. At this time co-operation between OX22$^+$ and OX22$^-$ helpers in producing worm rejection cannot be ruled out. However, this reaction is felt to be unlikely as recent results indicate that as few as 3×10^7 dividing R/I Day 2 cells transfer as much protection as 2×10^8 Day 3 efferent TDL (C. H. Wang and R. G. Bell, unpublished data). At this time no OX22$^+$ helpers are dividing and total contamination by OX22$^+$ cells is less than 3×10^6 cells.

The OX8$^-$ OX22$^-$ cells that transfer protection in these experiments were generated in the intestine very promptly after exposure to antigen in the form of infection with Ts. The early restriction of the ensuing blast cell response to the OX8$^-$ OX22$^-$ T-helper cell subset during the first 48 hr was unexpected. This may be dependent upon the site of antigen presentation, which is

presumably related to the preferred intracellular habitat of Ts in the epithelium of the crypts and lower 1/3 of the villus (Wright, 1979). This micro-anatomic position might favour stimulation of lymphoid cell populations outside the Peyer's patches during the initial infection. If protective cells are indeed stimulated locally at the site of residence of the adult worm, then the primary role of the lymph-borne protective blast cells may simply be their dissemination to non-parasitized areas of the intestine. However, the strong anamnestic response shown by rats depleted of migratory $OX8^-$ $OX22^-$ blasts also suggests that a major protective role is undertaken by the intestinally resident cells in addition to the effects of the migratory cells. A similar mucosal dissemination of IgA-secreting B-cell plasmablasts originating in the Peyer's patches and mesenteric nodes has been well documented (McWilliams, Phillips-Quagliata & Lamm, 1977; Craig & Cebra, 1971; Rudzik *et al.*, 1975). It has also been shown that IgA precursor cells proliferate locally and do not have to migrate in order to populate the lamina propria with IgA secreting cells (Mayrhofer & Fisher, 1979). The mucosal IgA system thus comprises both sedentary local proliferative elements as well as migratory constituents analogous to the protective local and migratory T-helper cell populations proposed here.

The mechanism by which the $OX8^-$ $OX22^-$ T-helper cells promote worm rejection is currently unknown; the induction of B-cell differentiation and antibody secretion is a known function of these cells (Mason *et al.*, 1983). Although recent experiments have shown that the target of the OX22 antibody, leucocyte common antigen, is lost when cells are activated (Powrie & Mason, 1988), it is not clear whether this applies to all T-helper cells in the rat. For example, we found dividing $OX22^+$ $OX8^-$ cells on Day 3 and have also found distinct functional attributes for the $OX22^+$ and $OX22^-$ T-helper subsets in TDL after Ts infection (C. H. Wang and R. G. Bell, unpublished observations). A role for B cells and antibody in rejection of Ts (Crum, Despommier & McGregor, 1977; Love, Ogilvie & McLaren, 1976) was suggested in passive and adoptive transfer experiments, although this conclusion has been challenged on the basis of experiments in mice where a role for inflammatory bone marrow-derived cells as the final effector has been proposed (Wakelin & Wilson, 1977; Wakelin & Donachie, 1981). The latter suggestion is buttressed by the persistent failure to effect primary worm rejection by the transfer of antibody alone. However, the evidence for inflammatory cells as the final effector is also indirect. Dissociation of the role of $OX22^-$ cells in promoting B-cell differentiation or inflammation in the gut is obviously important for understanding which T-helper functions are important for worm rejection.

The finding that Day 2 cells are responsive to both larval and adult worm antigens *in vitro* confirmed the specificity of these cells for antigens of the parasite and demonstrates the cells' potential for acting directly against adult worms. Specificity for adult Ts has previously been demonstrated in adult stage-specific infections after adoptive transfer of TD T-helper cell populations (Bell *et al.*, 1987). The cells present in R/I or efferent TD lymph 2 and 3 days after infection therefore have appropriate immune specificity to comprise the effector cell population that mediates worm rejection during the primary infection. Overall, the results demonstrate firstly that a cell subset $OX8^-$ $OX22^-$ within the T-helper cell population transfers protection against adult Ts in rats. Secondly, the experiments focus attention on the intestine as the site of generation of protective cells and the site at which protection is effected.

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