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Characterization of a cell population in thoracic duct lymph that adoptively transfers rejection of adult *Trichinella spiralis* to normal rats

R. G. BELL, M. KORENAGA & C. H. WANG *James A. Baker Institute for Animal Health, NYS College of Veterinary Medicine, Cornell University, Ithaca, New York, U.S.A.*

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SUMMARY

In *Trichinella spiralis*-infected rats, a population of cells in thoracic duct lymph (TDL) that can adoptively transfer protection to naive rats was identified and characterized. During the course of *T. spiralis* infection, blast cells appeared in lymph from Day 3–4, and only Day 3–4 TDL cells had protective properties after transfer. Protection was evident in a 1–2-day increase in the slow rejection of adult worms beginning 8–9 days after the challenge infection. The minimum number of TDL cells capable of transferring protection was $1{\cdot}8 \times 10^8$ cells. Transferred cells could protect against a challenge infection with adult worms alone. A double cross-over experiment demonstrated that major histocompatibility complex identity was essential for effective transfer of protection (MHC restriction). An experiment using the mitotic inhibitor vinblastine showed that the protective cells belonged to a dividing cell population. The phenotype of the protective TDL was confirmed by a two-step cell separation procedure. First, it was demonstrated that surface Ig^- cells (T cells) separated by affinity chromatography could transfer protection. Second, these surface Ig^- cells were divided into two subpopulations by panning using monoclonal antibodies OX-8 and W3/25. The results showed that $W3/25^+$ or $OX\text{-}8^-$ cells (T-helper) were effective in transferring protection. Protection was only seen when rats adoptively transferred with cells were challenged 1 day after cell transfer.

INTRODUCTION

In recent years several advances have been made in unravelling the steps that lead to rejection of *Trichinella spiralis* from inbred mice (Wakelin & Wilson, 1979; Grencis & Wakelin, 1982; Grencis, Reidlinger & Wakelin, 1985). During this period it has been shown that protection is a function of a population of dividing helper T cells (phenotypically $L3T4^+$) present in the mesenteric lymph node (MLN) from Day 4–8 after a primary infection (Wakelin & Wilson, 1977, 1979; Grencis *et al.*, 1985). More recently Reidlinger, Grencis & Wakelin (1986) demonstrated that T-helper cell lines stimulated *in vitro* with antigens of infectious muscle larvae could also transfer protection.

Passive transfer was also demonstrated in mice by Larsh, Goulson & Weatherly (1964) using peritoneal exudate cells, and in rats (Love, Ogilvie & McLaren, 1976; Crum, Despommier & McGregor, 1977; Despommier *et al.*, 1977) where protection was successfully transferred using antibody or affinity-purified B cells collected from hyperimmune donors. Although in these experiments both MLN cells (Love *et al.*, 1976) and TDL T cells (Crum *et al.*, 1977) were effective, the T lymphocytes were non-dividing and thought to be memory T cells that functioned by stimulating a B-cell antibody response (Crum *et al.*, 1977). Love *et al.* (1976) also concluded that MLN cells were effective due to the transfer of antibody production, although this was not tested directly. Larsh *et al.* (1964) used peritoneal exudate cells collected after the primary infection had been cleared. Due to the method of immunization, or time of harvest of cells, each of these experimental procedures therefore examined processes that were temporally more akin to what would be expected during a secondary or challenge infection. In addition, the rat experiments were unique in suggesting that antibody-forming cells were the major effector of adult worm rejection and, in this, contrast noticeably with the more extensive murine experiments described above.

The events of a primary infection were most closely followed by the experiments of Wakelin's group who primarily examined MLN cells collected 8 days after infection as they were more efficient than Day 4 cells (Wakelin & Wilson, 1977, 1979; Grencis & Wakelin, 1982). This is a late collection period in the primary infection as rejection begins by Day 9/10 in the NIH mice used in this experiment. Recipients of Day 8 immune MLN cells do not show worm rejection until a further 8 days after their infection with *T. spiralis*. A delay of this magnitude would not be expected if the cells collected on Day 8 were functional for

Correspondence: Dr R. G. Bell, James A. Baker Institute for Animal Health, NYS College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, U.S.A.

rejection at Day 9/10 in the primary cell donors. This fact was recognized by the authors who postulated that transferred T-helper cells stimulate an inflammatory granulocytic effector cell reaction (Wakelin & Wilson, 1980). In addition, the development of mesenteric T-cell lines capable of transferring protection under very similar conditions to the Day 8 MLN cells was achieved using muscle larvae antigens as the stimulant. These antigens are either not expressed or only very weakly expressed by adult worms (Parkhouse & Clark, 1983; Silberstein & Despommier, 1984), and therefore are unlikely to be the target of expulsive immunity in the primary infection. It is known that adult worms or their antigenic products are quite immunogenic (Chipman, 1957; Gamble, 1985) and lead to specific adult worm rejection (Bell, McGregor & Despommier, 1979). These observations suggest that the Day 8 MLNC collected from mice may be transferring a secondary-type reaction specific for pre-adult antigens rather than a primary-type response specific for adult worm antigens. By extension, we can anticipate that there will be a population of adult antigen-reactive cells that are instrumental in eliminating the primary infection.

Our object in the experiments described here was to develop a system in rats in which the properties of protective cells functioning during the primary infection could be examined. We chose the rat because it is considerably easier to harvest thoracic duct cells in rats than in mice, and an examination of the cell population that migrated naturally to the intestine was essential if the processes leading to worm rejection during the primary infection were to be followed in detail. A further motivation was the fact that rats were the species in which antibody-forming cells had been implicated in rejection. Although this is at variance with the concept of a T-cell mediated induction of inflammation in the infected gut, it is not at odds with the demonstration that T cells and bone-marrow cells are required to reconstitute protection in irradiated mice (Wakelin & Wilson, 1980). The nature of the bone-marrow cells has not been defined and it could be either or both B-cell precursors and/or inflammatory cells.

MATERIALS AND METHODS

Rats

Male or female AO ($RT1^u$), PVG ($RT1^c$) and AO/PVG ($RT1^u$) rats 6–10 weeks of age were used as appropriate. Most experiments were conducted with rats of the AO strain. Rats were bred at the Baker Institute vivarium and given food and water *ad libitum*.

Trichinella spiralis

The parasite was maintained by serial passage in retired breeder rats of DA, PVG or AO strains. The procedures used to isolate larvae, to infect rats and to count intestinal worms have been described before (Bell, McGregor & Adams, 1982; Bell & McGregor, 1980). Adult worms were obtained by removing the intestine of rats infected 4 days previously and incubating the opened, washed intestine in 0·85% saline for 1·5 hr at 37°. After this time the intestine was removed and emigrant worms collected by sieving through a 200-mesh screen. After counting to adjust the worms to the desired concentration, a volume of 1 ml was injected into the upper duodenum through a laparotomy incision.

Cells

Thoracic duct lymph (TDL) was collected at room temperature for 16–24 hr into heparinized Ringer solution without antibiotics. Cells were washed twice before adjustment to 2×10^7/ml in Hanks' balanced salt solution 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 to remove B cells, characterized by a high surface immunoglobulin content, from the TDL cell population (Crum & McGregor, 1976). Columns were prepared by coupling affinity-purified rabbit (or sheep) anti-rat $F(ab')_2$ to cyanogen bromide-activated Sephadex G-200 (Pharmacia, Uppsala, Sweden). Approximately 100 ml of swollen, coupled gel were poured into 50 mm diameter columns. After extensive washing, 100 ml of lymphocytes at a concentration of $1–2 \times 10^7$/ml were added dropwise to each column. The columns were washed with 450 ml of Hanks' BSS containing 5% fetal bovine serum. Non-adherent (surface Ig^- cells) were collected in the effuent. In order to elute adherent (surface Ig^+ cells), the columns were washed with Hanks' BSS containing 20% normal rat serum.

Separation of cell subsets

Recovered T cells were further fractionated using monoclonal antibodies W3/25 [specific for the rat T-helper cell equivalent of L3T4 (Barclay, 1981)] or OX-8 [specific for the rat cytotoxic/suppressor cell (Brideau *et al.*, 1980)] (Accurate, Westbury, NY) using the panning method described by Chen-Woan, Sajewski & McGregor (1985). Purified T cells obtained from affinity columns were incubated for 20 min at 4° in RPMI-1640 containing a 1:100 dilution of W3/25 or OX-8 antibody. After two washes they were incubated for 30 min at room temperature in petri-dishes coated with affinity-purified goat anti-mouse IgG. The latter comprised a mixture of 10% affinity purified goat anti-mouse IgG in normal goat IgG. Non-adherent cells were poured off after gentle rotation of the petri-dish for 30 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 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).

[^{3}H]thymidine uptake in vitro

In order to assess [^{3}H-]thymidine uptake, freshly collected TD lymphocytes were fractionated by panning (Chen-Woan *et al.*, 1985). The non-adherent T cells were 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. The results are expressed as counts per minute (c.p.m.) per 10^7 lymphocytes.

Vinblastine treatment

Vinblastine (Sigma, St Louis, MO) was dissolved in RPMI-1640

containing 1% normal rat serum and injected intravenously in a single dose equivalent to 3 μg/g body weight 15 hr before cannulation (McGregor & Logie, 1973). The presence of blast cells in vinblastine-treated TDL was assessed cytologically on stained smears. The frequency of blast cells in TDL from vinblastine-treated rats was 0·3%, whereas that from non-treated rats was 7·3%. Viability was more than 98% in both cases at the time of cell transfer.

Statistics

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

RESULTS

Appearance of surface Ig⁻ lymphoblasts in TDL

Rats were infected with 2000 muscle larvae (ML), and groups of two or three were cannulated at intervals thereafter. Collected cells were fractionated into surface Ig^+ and Ig^- populations, and were then incubated in [^{3}H]thymidine to assess uptake. The data, shown only for Ig^- cells (T-cell population), demonstrate that blast cells appeared in lymph very briefly, for approximately 24 hr, from Day 3–4 (Fig. 1).

Transfer of protection with TDL collected on various days

Prior to the discovery of the time of blast cell appearance in the thoracic duct, TDL transfers had been attempted with cells collected on Days 5–10 after infection without achieving protection. In order to determine whether TDL blasts could transfer protection, we examined the properties of this cell population. The number of cells transferred was based on one donor rat to one recipient rat and therefore varied depending on the cell recovery that was achieved. The protective capacity of TDL cells collected 3 or 4 days after infection of donor animals is shown in Table 1. Cells collected on Day 4 were capable of transferring protection, but this protection was usually weaker than that seen with Day 3 cells, and occasionally cells were not protective at all (data not shown). As mentioned above, cells collected from 5 days onwards never transferred protection (data not shown). In Fig. 2 we show that the time of terminal rejection is advanced by 1–2 days in rats receiving Day 3 TDL lymphocytes. The establishment of larval worms in the intestine was not affected by cell transfer (data not shown).

Table 1. The transfer of immunity with thoracic duct lymphocytes collected 3 or 4 days after *Trichinella spiralis* infection

	Day of donor cannulation*	Cells transferred†	Day of assay‡	Worm burden§	*P*
Exp. 1	3	$6·5\times10^8$	9	71 ± 26	<0·025
	—	—	9	409 ± 116	
Exp. 2	4	$5·8\times10^8$	8	262 ± 34	<0·0005
	4	$5·8\times10^8$	10	105 ± 51	<0·0005
	—	—	10	452 ± 27	

* Donor rats were infected with 2000 muscle larvae (ML) on Day 0.

† Cells were collected for 16–24 hr.

‡ Recipient rats were challenged with 1000 ML 1 day after cell transfer.

§ Data represent the means ± 1 SE of five rats per group.

Time of challenge of cell recipients

During these experiments we discovered that protection was not observed if the challenge infection was given on the same day as the cell transfer. A typical experiment demonstrating this is shown in Fig. 3. In this experiment Day 3 TDL were transferred to three groups of recipients, one of which was challenged with 1000 *T. spiralis* ML on the day of cell transfer, the others 1 day or 2 days later. Each group and its non-transferred control were killed 9 days after the infection. Protection was only seen in rats that were challenged 1 day after cell transfer.

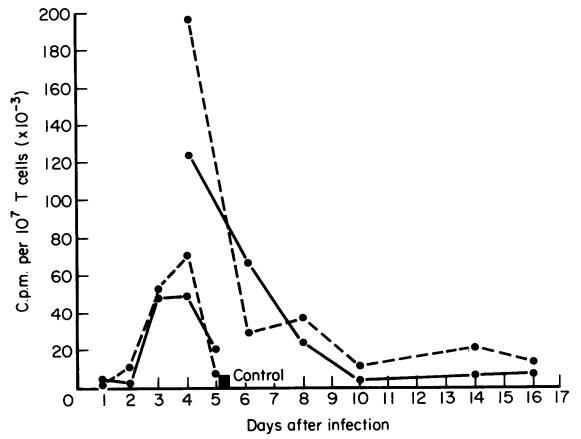

Figure 1. Kinetics of appearance of blast cells in thoracic duct lymph as measured by [^{3}H]thymidine uptake in freshly collected cells. Two separate experiments are shown, in each of which rats were infected with 2000 ML (—) or 5000 ML (– – –). Separation of T cells was performed by panning using rabbit anti-rat $F(ab')_2$ and a goat anti-rabbit IgG in the petri-dish. Infection occurred on Day 0.

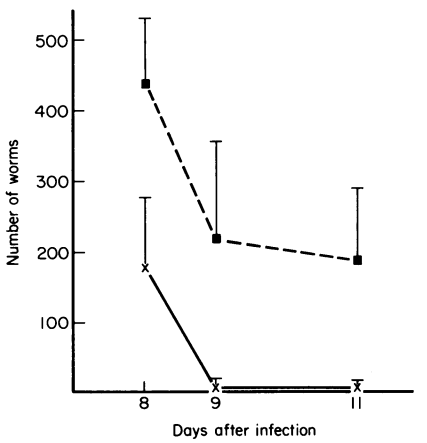

Figure 2. Kinetics of rejection of adult worms in rats given on Day 3/4 TDL from infected donors. Recipients were killed on Days 8, 9 and 11 after worm infection. Transfused cells produce an earlier rejection. Cell recipients (×—×), controls (■– – –■).

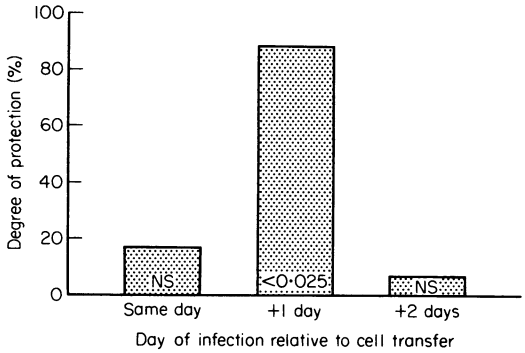

Figure 3. Effect of infection of adoptively transfused rats on the same day as cell transfer, 1 day later or 2 days later. All groups were assayed for intestinal worm burden 9 days after infection. Results were expressed as the degree of protection by the formula:

$$\text{degree of protection} = 1 - \frac{\text{worm counts in cell recipients}}{\text{worm counts in control}} \times \frac{100}{1}.$$

Rejection only takes place when infection follows cell transfer 1 day later.

Influence of infection size and cell dose on transfer

In order to determine whether infection with 2000 ML produced optimum reactivity in TDL, groups of cell donors were infected with 500, 2000 or 5000 ML prior to cannulation. The collected cells were transferred at a ratio of 1 rat equivalent/recipient. Although more worms were rejected at 9 days with increasing worm burden of the donor rat, the trend was not quite significant (Table 2). In order to determine the minimum number of cells capable of transferring protection, individual rats were transfused with $4{\cdot}5 \times 10^8$, $1{\cdot}8 \times 10^8$ or 1×10^8 TD lymphocytes collected from rats cannulated on Day 3 after infection. Worm rejection was identical in groups receiving 4·5 or $1{\cdot}8 \times 10^8$ cells (86 ± 106 or 68 ± 108, respectively) but rats receiving 10^8 cells were not different from controls (230 ± 77 and 290 ± 89, respectively).

Table 2. Influence of size of infection on transferability of immunity

Size of infection*	Cells transferred†	Worm burden‡	*P*
500 ML	$5{\cdot}2 \times 10^8$	124 ± 23	<0·005
2000 ML	8×10^8	75 ± 26	<0·005
5000 ML	8×10^8	30 ± 21	<0·005
—	—	389 ± 46	

* Donor rats were infected on Day 0 and cannulated on Day 3.

† Cells were transferred on the basis of one rat equivalent (one cannulated rat → one recipient rat). The transferred cell number after an infection with 500 muscle larvae reflects lower cell output from these rats.

‡ Recipient rats were challenged with 2000 ML 1 day after cell transfer. Worm burdens were assessed 9 days after the challenge infection. Data represent the means ± 1 SE of five rats per group.

Specificity of transferred cells

It was important to determine whether transferred TDL lymphocytes could protect against a challenge infection with adult worms alone. This would suggest that these cells could indeed function during the primary infection to mediate adult worm rejection. To do this, TDL were collected 3 days after infection and transferred to two groups of rats. One day later one group was infected orally and the second group had 1500 4-day-old adult worms transferred directly into the small intestine via a laparotomy. The results showed that as many adult worms were rejected by Day 9 in rats receiving adult worms as in rats infected with muscle larvae (Table 3).

Analysis of the cell population conferring protection

In order to determine whether MHC compatibility with the host was essential for function, cells were transferred in a double cross-over experiment to MHC compatible and incompatible donors. Both AO ($RT1^u$) and PVG ($RT1^c$) rats served as donors, and cells were transferred into AO, PVG and PVG-AO (PVG background AO MHC-$RT1^u$) congenic rats. The results showed that compatibility at the MHC was essential for effective transfer (Fig. 4).

Table 3. Effectiveness of transferred cells in expressing immunity against adult worms

Cells transferred*	Challenge with:†	Worm burden‡	*P*
$4{\cdot}5 \times 10^8$	1500 adult worms	40 ± 28	<0·005
—	1500 adult worms	358 ± 60	
$4{\cdot}5 \times 10^8$	1000 ML	86 ± 37	<0·01
—	1000 ML	290 ± 40	

* Donor rats were infected with 2000 ML and cannulated on Day 3 after infection.

† Challenge was done 1 day after cell transfer.

‡ Worm burden was assessed 9 days after the challenge infection. Results represent the means ± 1 SE of five rats per group.

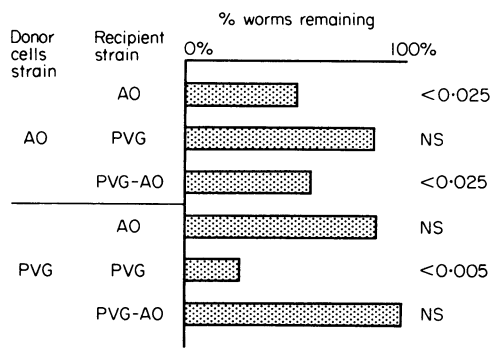

Figure 4. MHC restriction of adoptive transfer of worm rejection from the intestine. Donor rats of two distinct strains were used [AO ($RT\text{-}1^u$) and PVG ($RT\text{-}1^c$)] and cells were transferred into AO^u PVG^c and PVG-AO^u recipients, respectively. Results are expressed as the percentage of worms remaining (compared with controls of the same strain not receiving cells) at harvest on Day 9.

The possibility that the protective cells belong to a dividing cell population was tested by injecting vinblastine into Day 2-infected rats. On Day 3 the thoracic duct was cannulated, and the cells collected overnight were transferred intravenously into recipients. The recipients were challenged *per os* with 1000 ML the day after cell transfer. Lymphocytes from vinblastine-treated rats were unable to transfer immunity (intestinal worms 9 days after transfer: vinblasting-treated donors 236 ± 69, untreated donors 13 ± 6, non-transferred control 251 ± 43). This result suggests that large dividing lymphocytes are the effector cells responsible for mediating host protective immunity against *T. spiralis*.

In order to confirm further the presumptive T-cell nature of the transferred cells, fractionation on Sephadex G-200 coupled with rabbit anti-rat $F(ab')_2$ was used to separate surface Ig^+ cells from surface Ig^- cells. Recipient rats were infused with 1 rat equivalent of each cell subpopulation which comprised 3×10^8 surface Ig^- cells and $1{\cdot}5 \times 10^8$ surface Ig^+ cells. Positive control rats were infused with unfractionated cells. At harvest, 9 days after infection, only those rats receiving surface Ig^- cells (T cells) or whole TDL displayed enhanced rejection (Table 4).

The T-cell subpopulation of TDL was further fractionated into its two major subsets as defined by monoclonal antibodies W3/25 (T-helper cells) or OX-8 (cytotoxic/suppressor cells). This separation was achieved by subjecting the affinity-purified T-cell population to panning after incubation of the cells in either OX-8 or W3/25 antibody. In order to ensure that the results were not biased by a particular monoclonal antibody the experiment was done in both directions; i.e. when $OX\text{-}8^+$ cells were positively selected using OX-8 antibody (Table 5) and when $W3/25^+$ cells were selected with W3/25 antibody (Table 6). The results were consistent and showed that the cell population that failed to adhere with OX-8 or that was identified by W3/25 conferred protection.

Table 4. Transfer of immunity after fractionation of thoracic duct lymphocytes into surface Ig^- and Ig^+ subpopulations

Cells transferred*	Worm burden†	*P*
3×10^8 sIg^-	59 ± 32	$<0{\cdot}005$
$1{\cdot}5 \times 10^8$ sIg^+	369 ± 87	NS
$4{\cdot}5 \times 10^8$ unfractionated	129 ± 35	$<0{\cdot}025$
—	474 ± 103	

* Donor rats were infected with 2000 ML and cannulated on Day 3. Thoracic duct lymphocytes collected on Day 4 were fractionated into surface Ig^+ and surface Ig^- cells by passage through an affinity column of Sephadex G-200 to which rabbit anti-rat $F(ab')_2$ had been coupled. The surface Ig^- subpopulation had 10% contamination with surface Ig^+ cells, and the surface Ig^+ subpopulation was contaminated with 4% surface Ig^- cells. Recipient groups were infected with 1000 ML 1 day after cell transfer and harvested for worm counts 9 days later.

† Mean ± 1 SE of five rats per group.

Table 5. Transfer of immunity after fractionation of thoracic duct T lymphocytes into $OX\text{-}8^+$ and $OX\text{-}8^-$ subpopulations

Cell population transferred*	Worm burden†	*P*
$2{\cdot}7 \times 10^8$ T-TDL	73 ± 23	$<0{\cdot}01$
$2{\cdot}9 \times 10^8$ $OX8^-$	92 ± 33	$<0{\cdot}01$
$2{\cdot}1 \times 10^8$ $OX8^+$	258 ± 83	NS
—	351 ± 35	

* Donor rats were infected with 2000 ML and cannulated on Day 3.

† Recipient rats were challenged with 1000 ML the day after cell transfer and worm burden was assessed on Day 9 after the challenge infection. Mean ± SE from four rats per group.

Table 6. Transfer of immunity after fractionation of thoracic duct T lymphocytes into $W3/25^+$ and $W3/25^-$ subpopulations

Cell population transferred*	Worm burden†	*P*
$2{\cdot}7 \times 10^8$ T-TDL (T-cell-enriched)	95 ± 87	$<0{\cdot}05$
$1{\cdot}9 \times 10^8$ $W3/25^+$	167 ± 37	$<0{\cdot}05$
$1{\cdot}1 \times 10^8$ $W3/25^-$	381 ± 56	NS
—	343 ± 81	

* Donor rats were infected with 2000 ML and cannulated on Day 3.

† Recipient rats were challenged with 1000 ML on the day after cell transfer, and worm burden was assessed on Day 9 after the challenge infection. Mean ± SE from four rats per group.

DISCUSSION

The results reported here show that a population of T cells in thoracic duct lymph of *Trichinella spiralis*-infected rats, identified by the W3/25 cell surface marker, can transfer protection to naive rats. Rejection of worms after adoptive transfer takes place with the slow kinetics of disappearance characteristic of rejection of a primary infection. Adult worms alone are rejected and there is no requirement for exposure to larvae or pre-adult antigens for rejection to occur. The results were unusual in two respects: first, the cell population conferring protection is present in the lymph for a very short period, especially in view of the time–course of the infection; second, it is necessary to infect cell-recipient rats the day after cell transfer to achieve rejection.

Overall, the results of this investigation bring the protective mechanism of the rat much more into line with the by now well-defined cellular components of rejection of *T. spiralis* described in the NIH mouse (Grencis *et al.*, 1985; Grencis & Wakelin,

1982; Wakelin & Wilson 1977, 1980). Points of agreement include the phenotype of transferred effector cells (L3T4$^+$ Ly2$^-$) in the mouse and W3/25$^+$ in the rat. Both cell types have been extensively characterized as helper T cells. In both cases it is the actively dividing blast cells that confer protection. Experiments in rats using the parasite *Nippostrongylus brasiliensis* have also shown that it is the T-cell population in TDL that is most active in transferring protection (Ogilvie *et al.*, 1977; Nawa, Parish & Miller, 1978). The crucial role of T cells has thus been established with several distinct systems. Of more interest in the experiments reported here is the transient appearance of protective T cells. Nawa & Miller (1978) working with *N. brasiliensis* in rats did not find protective cells before Day 10 although they only examined Day 5 TDL and may have missed an earlier peak. For our part, we have been unable to find protective cells as yet among TDL collected later than 4 days after infection.

While protective cells are only present in rat TDL for 24–36 hr, the equivalent population is only present in the MLN of NIH mice from Days 4–8 (Wakelin & Wilson, 1977; Grencis & Wakelin, 1982). It is possible, and in keeping with the development of memory, that the dividing cells present in the murine MLN from Days 5–8 are largely non-migratory memory cells. In other words, the natural effectors of rejection in mice may also appear in thoracic duct lymph at the same time as they do in rats. Unfortunately this cannot be directly confirmed because at this stage MLN cell transfer in rats does not work in our hands and TDL collection in mice has not been attempted.

The very early appearance of protective TDL blast cells is consistent with the long period taken (8–10 days) before rejection occurs in passive recipients of cells. This is seen in both the experiments reported here and those of Wakelin & Wilson (1977, 1979). As such, the time–course of appearance of protective cells in TDL is consistent with the initiation of rejection both in the cell donors and in the adoptive transfer recipients. Furthermore, the TDL blast cell has a pronounced tendency to localize in the gut (Despommier *et al.*, 1977), the site where rejection is actually mediated. The fact that the 3-day helper blast cells we have described here will also reject adult worms reinforces the view that these cells are functionally involved in the process of rejecting the primary infection in the donor rats.

The results appear to be more at odds with the previous work using *T. spiralis* of Love *et al.* (1977) as well as the *N. brasiliensis* study of Nawa *et al.* (1978). All of these groups implicated antibody or B cells in protection: Love *et al.* (1976) by direct transfer of serum, whereas Crum *et al.* (1977) and Nawa *et al.* (1978) showed that B cells in thoracic duct lymph conferred protection. Both cell transfer studies used rats that had received multiple immunizations, and it is possible that the effectors they were transferring were influenced by this. For example, antibody may have a more important protective role in a challenge infection. Crum *et al.* (1977) did find that a non-dividing T-cell population could also transfer immunity, and the authors suggested that these cells may well have had a helper role for antibody production.

It is worth noting that the T-helper cells found in mice during the primary infection are not believed to be the mediators of rejection (Wakelin & Wilson, 1980). A second population of effectors has been postulated to be induced by these helper cells. Experiments conducted in irradiated mice have shown that bone-marrow cells are required for immune MLN helper cells to be effective (Wakelin & Wilson, 1980). The bone-marrow cells have never been phenotypically defined and, although circumstantial evidence points to myeloid cells, the possible role of B cells or NK cells has not been excluded in the murine system. In direct experiments examining the role of B cells isolated from immune MLN, activity was usually found (Wakelin & Wilson, 1979) and, although this could be abolished or reduced by treatment with anti-Thy 1.2, dose–response experiments were not conducted. It is to be expected that larger numbers of B cells may be required to transfer protection in the absence of T-cell amplification.

We believe that a role for either B or NK cells is at least as consistent with bone-marrow data as the currently accepted role for inflammatory granulocytic cells. If this were so, then essentially all experimental results using adoptive transfer of protection with *T. spiralis*- or *N. brasiliensis*-infected rodents would be mutually supportive. That is, the T-helper cell stimulates an antibody-producing cell (or perhaps an NK cell) to react against adult *T. spiralis*. Either could constitute the as yet undefined bone-marrow requirement demonstrated by Wakelin & Wilson (1980). This proposal would not necessarily preclude an additional role for inflammatory granulocytic cells as was suggested by Wakelin & Wilson (1980). There is as yet no unequivocal evidence for any single terminal effector of adult worm rejection, and more than one mechanism remains a distinct possibility.

The most peculiar finding and our biggest initial obstacle was the fact that protection was not observed when rats were challenged on the same day as cell transfer. We have no explanation for this as yet, but preliminary evidence is consistent with altered migratory properties of the transferred cells when infection occurs concurrently. It seems likely that functional properties are vested specifically in the TDL helper cells that enter the intestine. Intestinal worm implantation may well interfere with this process and hence is likely to upset or delay the developing intestinal reaction and prevent worm rejection. In any case the identification of a protective cell population that naturally migrates to the intestine, and the restricted conditions under which protection is expressed, suggests that this system can provide insights into the intestinal immune processes that lead to worm expulsion.

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