Immunology 1977 33 787

Immunity to *Trichinella spiralis*

I. TRANSFER OF RESISTANCE BY TWO CLASSES OF LYMPHOCYTES

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Received 2 *December* 1976; *accepted for publication* 5 *April* 1977

Summary. Rats can be solidly immunized against *Trichinella spiralis* by a series of methyridine-terminated oral infections with *T. spiralis* larvae. Injections of thoracic duct lymphocytes (TDL) obtained from such animals can protect normal rats against a *Trichinella* challenge. The protective cells belong to two populations which differ with respect to their adherence to affinity columns prepared with rabbit antibody to rat $F(ab')_2$. Immune lymphocytes in the column-adherent B cell fraction are inhibited by vinblastine, whereas those in the non-adherent, T cell fraction are resistant to this drug. The above observations suggest that acquired resistance to *T. spiralis* is mediated by two classes of lymphocytes: B cells which are delivered to the thoracic duct and hence to the blood while still in active cycle, and T cells which have a potentially long life-span and presumably belong to a pool of recirculating small lymphocytes.

INTRODUCTION

Animals that recover from a primary infection with the nematode, *Trichinella spiralis*, develop a measure of resistance to re-infection (Ducas, 1921; McCoy, 1931). While it is firmly established that immunity to *T. spiralis* can result from exposure to either larval stage or adult stage antigens (Zaiman, Wilson, Rubel & Stoney, 1954; Chipman, 1957; Denham, 1966), the protective mechanisms operative against the parasite are poorly understood. Recent studies (Love, Ogilvie & McLaren, 1976) using transfer techniques demonstrated that rats that have experienced both the enteral and parenteral phase of the infection were capable of transferring significant immunity to naive recipients with their cells, serum, or a combination of both. But the cells that transfer immunity have not been identified, nor has the development of resistance in infected animals been related to the appearance in the serum of a particular class(es) of protective antibodies.

The problem has been investigated in (Lewis × DA)F_1 hybrid rats using transfer techniques, relatively pure populations of lymphocytes and an immunization protocol that favors induction of immunity primarily to the larval stage antigens of the enteral phase of the infection. We will demonstrate that the thoracic duct lymph of rats stimulated by 4 abbreviated infections with *T. spiralis* contains two populations of lymphocytes that have protective properties. One population segregates with T cells on affinity columns prepared with rabbit antibody to rat $F(ab')_2$. The cells in this fraction divide infrequently and presumably are small lymphocytes.

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The second population of protective lymphocytes segregates with B cells and is made up of lymphocytes that are in active cycle.

MATERIALS AND METHODS

Animals

Male (Lewis × DA)F_1 hybrid rats were used. Cell donors weighed 200–300 g whereas recipients weighed 150–200 g.

Trichinella spiralis

T. spiralis larvae were isolated from the skeletal muscles of rats which had been infected for longer than 4 weeks (Despommier, 1971). Larva donors were killed by cervical dislocation. The skinned and eviscerated carcasses were then minced in a meat grinder and digested for 1 h at 37° in distilled water (25 ml/g) containing 1% (w/v) pepsin (Fisher Scientific) and 1% (v/v) hydrochloric acid. Larvae were isolated by passing the digestion mixture through a 200 mesh screen. The larvae were backwashed from the screen, sedimented by centrifugation (200 *g* × 5 min), then resuspended in 0·6% (w/v) nutrient broth (Difco Laboratories, Detroit, Mich.) containing 2% (w/v) gelatin.

Immunization of rats and measurement of protective immunity

Unanaesthetized rats were immunized with 200 viable *T. spiralis* larvae. The latter were drawn from a spinner flask into a 1 ml syringe and administered orally through an 18 gauge feeding needle (Popper & Sons, Inc., New Hyde Park, N.Y.). Forty-eight h later, the infection was terminated by injecting the animals subcutaneously with 300 mg/kg of methyridine (Denham, 1965). This procedure was repeated 4 times, at weekly intervals. The immunity engendered in these actively immunized animals, and in recipients of their cells, serum, or lymph, was measured by enumeration of total body muscle larvae 30 days after oral challenge with 700 viable *T. spiralis* larvae (Despommier, 1971). The level of protection was expressed as percent difference in mean number of larvae in the muscles of 5 test subjects and 5 non-immunized controls.

Preparation of cells

Cells for adoptive transfer were taken from rats 6–7 days after the last of 4 immunizing infections. Thoracic duct lymphocytes (TDL) were obtained from rats incannulated on day 6. The cells were collected for 16–24 h into heparinized Ringer's solution without added antibiotics. Cells were expressed from the spleen and from mesenteric lymph nodes by teasing the tissues in Hanks's balanced salt solution (HBSS) containing 2 units of heparin/ml. Peritoneal exudate cells (PEC) were obtained 24 h after the animals had been stimulated intraperitoneally with 50 μg of alcohol-killed *Listeria monocytogenes*. The technique for harvesting PEC, and the cellular composition of such exudates, have been described elsewhere (Koster, McGregor & Mackaness, 1971).

Cells were sedimented by centrifugation (500 *g* × 10 min), washed once in HBSS containing 1% foetal calf serum (FCS) and resuspended in fresh medium at a concentration suitable for intravenous injection.

Fractionation of TDL by affinity chromatography

A chromatographic procedure described elsewhere (Crum & McGregor, 1976) was used to separate rat TDL into two fractions which coincide or closely overlap with T and B cells. In brief, $1{\cdot}5 \times 10^9$ to 2×10^9 TDL in 100 ml of medium 199 were applied to a column of 100 ml of Sephadex G-200 (Pharmacia, Uppsala, Sweden) to which 30–35 mg of rabbit anti-rat $F(ab')_2$ had been covalently linked. Non-adherent cells were recovered by washing the column with 450 ml of HBSS containing 5% FCS. The great majority of non-adherent TDL lacked demonstrable surface immunoglobulin (Ig) and were scored as T lymphocytes. Adherent cells were then eluted from the column with 400 ml of 20% normal rat serum in HBSS. The majority of adherent TDL had readily demonstrable surface Ig and were scored as B lymphocytes.

The proportion of Ig-bearing cells in each fraction was determined by indirect immunofluorescence using rabbit anti-rat $F(ab')_2$ diluted 1 : 16 and a fluorescent goat anti-rabbit Ig (Cappel Laboratories, Downingtown, Pa.) diluted 1 : 25 (Crum & McGregor, 1976). Lymphocytes with detectable Ig accounted for 1–5% in the non-adherent fraction, 94–98% in the adherent fraction, and 27–31% in the unfractionated lymph of actively immunized rats.

Functional markers of T and B cells

Acquired resistance to *L. monocytogenes* is mediated by newly formed T cells, many of which are in

active cycle (Crum & McGregor, 1976). Their activity was determined indirectly, by measuring the level of specific antimicrobial resistance conferred on recipient rats by an intravenous injection of immune TDL (McGregor, Koster & Mackaness, 1971).

Memory of the bacteriophage ϕX 174 is vested in non-dividing small lymphocytes which adhere to anti-rat F(ab')$_2$ columns (Crum & McGregor, 1976). TDL obtained from specifically immunized donors were transferred intravenously into normal recipients which were challenged by the same route with 10^{10} plaque forming units of ϕX. The level of phage neutralizing antibody in the serum 6 days after challenge was taken as a measure of B cell memory.

*Treatment of lymphocytes with vinblastine**

TDL were suspended at a density of 10^7 cells/ml in medium 199 containing 5% FCS, 1 unit of heparin/ml, 60 units of penicillin/ml, and 6 μg of streptomycin/ml. Vinblastine was added to a final concentration of either 0·1 μg/ml or 1·0 μg/ml. The TDL were incubated *in vitro* at 37° for 10 h. Immediately thereafter, the cells were washed in HBSS containing 1% FCS, viability was determined by dye exclusion and the cells were resuspended at a density of 10^8/ml, based upon the cell count prior to incubation.

Statistical analysis

Differences between means of paired groups were evaluated by Student's-'*t*' test. When more than two groups were compared, analysis of variance was applied and differences between means were evaluated by the Q test (Snedecor & Cochran, 1972).

RESULTS

Resistance in actively immunized rats

Table 1 indicates that rats can be solidly protected against *T. spiralis* by a series of 4 methyridine-abbreviated infections. The drug itself had no influence on the challenge infection as evidenced by the similar numbers of *T. spiralis* larvae present in the muscles of methyridine-treated rats and untreated controls.

Transfer of resistance by TDL

Love, Ogilvie & McLaren (1976) demonstrated that mesenteric lymph node cells (MLC) from specifically immunized donors can protect normal rats against a challenge infection with *T. spiralis*. We, too, measured the protective properties of MLC. In addition, the following cells were assayed: spleen cells, PEC and TDL. All conferred a measure of resistance; however, TDL were the most potent, and they alone transferred significant immunity in all experiments.

The results of eight separate experiments using *T. spiralis*-immune TDL have been combined in Fig. 1. It can be seen that there was a dose-response relationship between the number of lymphocytes transferred and the level of resistance in recipient rats.

Whereas living TDL from specifically immunized donors conveyed substantial immunity to an oral *T. spiralis* challenge, heat-killed TDL from the same donors did not protect (Table 2). This finding indicates that acquired resistance to the parasite cannot be ascribed to active immunization of the animals by heat-stable *Trichinella* antigens which were carried into the recipients at the time of cell-transfer.

Failure to transfer resistance against *T. spiralis* with either immune serum or lymph

There have been several reports (Culbertson, 1942;

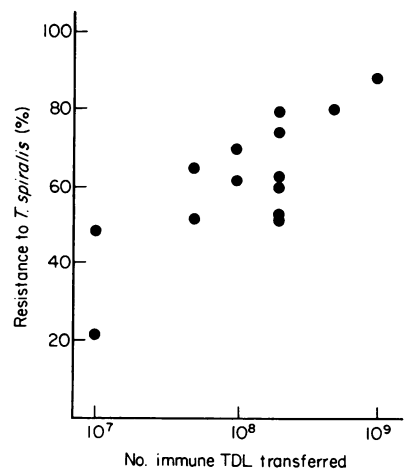

Figure 1. Plots depicting the immunity to *T. spiralis* conferred on normal rats by an intravenous injection of TDL obtained from specifically immunized donors. Each plot represents the percent difference in number of larvae in the tissues of 5 adoptively immunized subjects and 5 normal, non-immunized controls that were challenged orally with 700 larvae. The level of acquired resistance was proportional to the number of lymphocytes transferred.

* Vinblastine sulphate (Velban) was a gift from Eli Lilly and Co., Indianapolis, Ind.

Table 1. Immunity to *T. spiralis* in actively immunized rats*

Treatment of rats	Challenge infection†	Muscle larvae‡
Immunized	+	1250 ± 957
Immunized	–	700 ± 837
Methyridine§	+	43,000 ± 5291
Normal	+	41,400 ± 5319

* Animals infected orally with 2000 *T. spiralis* larvae were injected 48 h later subcutaneously with 300 mg/kg of methyridine. The procedure was repeated 4 times at weekly intervals.

† Rats were infected orally with 700 *T. spiralis* larvae 7 days after the last immunizing infection.

‡ Larva burden 30 days after challenge. Means of 5 ± s.d.

§ Treatment with methyridine only.

Table 2. Transfer of resistance to *T. spiralis* by living TDL

Inoculum*	Muscle larvae†	Protection‡ (%)	*P* value
Living TDL (2×10^8)	24,600 ± 4878	52	< 0·05
Heat-killed TDL§ (2×10^8)	61,600 ± 19,970	0	

* Obtained from donor rats 6–7 days after the last of 4 methyridine-abbreviated infections. The cells were injected intravenously into normal recipients that were challenged orally with 700 *T. spiralis* larvae.

† Means of 5 ± s.d.

‡ Percent difference 30 days after challenge, in muscle larvae in groups of 5 recipients and 5 non-immunized controls.

§ Heated for 1 h at 56°.

Love, Ogilvie & McLaren, 1976) that injections of specific immune serum can protect recipient rats against *T. spiralis*. But our own efforts to passively immunize rats with either serum or lymph were not successful.

Table 3 shows that a significant level of immunity was transferred by TDL but not by serum. Moreover, serum from immune rats failed to augment the resistance transferred by cells. In a separate experiment, also recorded in Table 3, resistance to *T. spiralis* was transferred by immune TDL, whereas an injection of concentrated cell-free lymph from the same immunized donors was devoid of protective activity. The latter finding virtually excludes the possibility that recipient rats were immunized by soluble antigens of the parasite and implies that if antibodies are involved in host resistance, their contribution depends upon the cells that make them or convey them in the circulation.

The cellular response to infection in individual rats

Substantial variation was observed from experiment to experiment in the level of immunity transferred by *T. spiralis*-immune TDL. The variation seems not to be related to a difference in the response of individual rats to the same series of drug-abbreviated infections. This conclusion was drawn from an experiment in which TDL were collected from 5 infected donors. The cells were *not* pooled, as they were in most

Table 3. Effect of serum, lymph or TDL derived from immune donors on resistance of rats to *T. spiralis*

Experiment	Inoculum*	Protection† (%)	*P* value
1	TDL (2×10^8)	64	< 0·001
	serum (2 ml)	22	n.s.§
	TDL (2×10^8) + serum (2 ml)	64	< 0·001
2	TDL (2×10^8)	53	< 0·001
	cell-free‡ lymph (2 ml)	0	

* Serum, lymph or TDL were obtained from *Trichinella*-immune rats, 6–7 days after the last of 4 methyridine-abbreviated infections. These were injected intravenously into normal recipients that were challenged orally with 700 *Trichinella* larvae.

† Percent difference 30 days after challenge, in muscle larvae in groups of 5 recipients and 5 non-immunized controls.

‡ Concentrated × 10.

§ n.s. = not significant.

Table 4. Capacity of TDL from individual rats to transfer immunity to *T. spiralis**

Immune donor	TDL collected $\times 10^{-6}$	No. recipients	TDL transferred $\times 10^{-6}$	Muscle larvae†	Protection‡ (%)
1	1046	4	200	24,500 ± 7517	68
2	754	3	200	34,670 ± 13,497	55
3	1094	4	200	26,500 ± 5500	65
4	828	3	200	22,700 ± 1734	70
5	1021	4	200	23,000 ± 3400	70
Normal	—	5	200	76,800 ± 7848	—

* TDL were collected from donor rats over a 24 h period 6–7 days after the last of 4 methyridine-abbreviated infections. 2×10^8 lymphocytes from individual donors were infused into normal recipients that were challenged orally with 700 *T. spiralis* larvae.

† There was no difference between test groups; however, all differed significantly ($P < 0{\cdot}01$) from the normal group.

‡ Percent difference 30 days after challenge, in muscle larvae in groups of 5 cell recipients and 5 non-immunized controls.

experiments. Instead, lymphocytes from each collection were infused into separate recipients (2×10^8/rat). The recipients, and 5 rats given 2×10^8 TDL from normal non-immunized rats, were challenged orally with 700 *T. spiralis* larvae. All were killed 30 days after challenge for enumeration of muscle larvae.

Table 4 shows that lymphocytes obtained from different immune donors transferred the same level of resistance.

Transfer of immunity by T cells and B cells

The presence of protective cells in the thoracic duct lymph of *T. spiralis* immune rats indicated that immunity to the parasite was mediated by lympho-

Table 5. Transfer of immunity to *T. spiralis* by TDL inocula enriched in either T or B cells

Experiment	TDL inoculum*	Lymphocytes transferred $\times 10^{-6}$ Total	Surface Ig positive	Surface Ig negative	Protection† (%)
1	Unfractionated	100	27·0	73·0	62
	T-cell-enriched	100	1·8	98·2	54
		200	3·6	196·4	71
	B-cell-enriched	100	96·0	4·0	64
2	Unfractionated	100	30·0	70·0	75
	T-cell-enriched	100	1·8	98·2	57
		200	3·6	196·4	75
	B-cell-enriched	100	97·0	3·0	56

* Obtained from rats immunized with *T. spiralis*. Cells fractionated on rabbit anti-rat $F(ab')_2$ columns were injected intravenously into normal rats. The latter were challenged orally with 700 *T. spiralis* larvae.

† Percent difference 30 days after challenge, in muscle larvae in groups of 5 cell recipients and 5 non-immunized controls. All differed significantly ($P < 0{\cdot}01$) from the control group.

cytes. However, the results leave open the question whether resistance is conveyed by T cells, B cells or both. Data obtained in experiments using class-enriched lymphocyte fractions gave a clue to the identity of the cells concerned. Column-purified fractions enriched in either T cells or B cells were infused in equal numbers into normal rats.

Table 5 shows that the separation procedure resulted in an approximate 20-fold reduction of Ig-bearing (B) cells in the fraction enriched T cells. Similarly, the B-cell-enriched fraction was severely depleted of T cells, i.e. lymphocytes that lacked readily demonstrable surface Ig. The two class-enriched TDL fractions had approximately the same protective capacity; on a cell-for-cell basis, each transferred approximately the same level of immunity against a *T. spiralis* challenge. The above finding takes on meaning when it is remembered that the level of resistance in adoptively immunized rats is proportional to the number of immune lymphocytes transferred (Fig. 1). Further evidence that both T cells and B cells have protective properties was obtained in an experiment in which vinblastine was used to inhibit the immunologic performance of dividing lymphocytes. However, it was first necessary to determine a concentration of the drug that can inhibit dividing lymphocytes without also inhibiting nondividing small lymphocytes.

Effect of vinblastine on dividing and nondividing lymphocytes

Rats were immunized with both *L. monocytogenes* and ϕX 174. The above agents were used, because they provoke the formation of antigen-activated lymphocyte populations that differ with respect to turnover, and thus afford an opportunity to evaluate the inhibitory properties of vinblastine. *L. monocytogenes* stimulates the formation of protective T cells, many of which are delivered to the thoracic duct while still in a blast condition (McGregor & Logie, 1973; Crum & McGregor, 1976). By comparison, a priming injection of ϕX leads to the imprinting of memory in B cells that divide infrequently or not at all (Gowans & Uhr, 1966; McGregor & Mackaness, 1975; Crum & McGregor, 1976). Thoracic duct lymph containing these two populations of activated lymphocytes were incubated *in vitro* at 37° for 10 h in the presence or absence of vinblastine. Thereafter the cells were washed and transferred intravenously into normal recipients. The latter were challenged by the same route with either $2{\cdot}32 \times 10^6$ *L. monocytogenes* or 10^{10} plaque forming units of ϕX 174.

Table 6 shows that vinblastine greatly diminished the protective power of *Listeria* committed lymphocytes; but the drug had no effect on ϕX memory cells. The above findings encourage the belief that low concentrations of vinblastine are selectively toxic for dividing lymphocytes. In any case, nondividing B cells are spared. The resistance of ϕX memory cells to vinblastine was exploited in the following experiment in which the drug was used to inhibit protective B cells in the thoracic duct lymph of *T. spiralis*-immune rats.

Table 6. Effect of vinblastine on TDL that transfer immunity to *L. monocytogenes* and those that carry immunological memory of ϕX 174

Vinblastine treatment of TDL (μg/ml)*	Acquired resistance to *L. monocytogenes* ($\log_{10}$ protection)†	Memory of ϕX 174 (Antibody titre)‡
0	2·01	132 ± 18
0·1	0·85	n.d.§
1·0	0·84	138 ± 13

* TDL obtained from donor rats 6 days after an immunizing infection with *L. monocytogenes* and 28 days after the animals had been given a priming injection of ϕX 174. The cells were incubated *in vitro* at 37° for 10 h.

† Mean difference 48 h after challenge in viable *Listeria* ($\log_{10}$) in the spleens of 5 adoptively immunized subjects given $1{\cdot}25 \times 10^6$ TDL/g and 5 non-immunized controls.

‡ Anti-ϕX antibody titre (k) in the serum 6 days after cell transfer (2×10^8 TDL/rat) and intravenous challenge with 10^{10} plaque forming units of ϕX 174. Means of 5 ± s.d. antibody titre in similarly challenged but unimmunized controls = 6·6 ± 1·7.

§ n.d. = not done.

Table 7. Effect of vinblastine on *T. spiralis*-immune lymphocytes which segregate with either T or B cells

TDL inoculum*	Culture conditions: Temperature (°)	Culture conditions: Vinblastine	Viability (%)	Memory of ϕX 174 (antibody titre)†	Immunity to *T. spiralis*: Protection‡ (%)	*P* value
Unfractionated	37	+	67		55	<0·01
	37	−	66	n.d.§	70	<0·01
	4	−	98		62	<0·01
T-cell-enriched	37	+	48		52	<0·01
	37	−	54	n.d.§	53	<0·01
	4	−	98		52	<0·01
B-cell-enriched	37	+	75	401 ± 60	7	n.s.
	37	−	85	412 ± 92	36	<0·01
	4	−	98	415 ± 123	40	<0·01

* TDL from rats immunized with *T. spiralis* were fractionated on rabbit anti-rat $F(ab')_2$ columns. The cells were then incubated *in vitro* for 10 h in the presence or absence of vinblastine (1 μg/ml). Immediately thereafter 2×10^8 TDL were infused into each of 5 normal recipients that were challenged orally with 700 *T. spiralis* larvae. The individual cell fractions were assayed on a cell-for-cell basis dependent upon their viability before cell transfer.

† Anti-ϕX antibody titre (k) in the serum 6 days after cell transfer and intravenous challenge with 10^{10} plaque forming units of ϕX 174. Means of 5 ± s.d. antibody titre in similarly challenged but unimmunized controls = 2·0 ± 0·6.

‡ Percent difference 30 days after challenge, in muscle larvae in groups of 5 cell recipients and 5 non-immunized controls.

§ n.d. = not done.

Effect of vinblastine on the mediators of resistance to *T. spiralis*

Adult rats were given a priming injection of ϕX 174. The animals were then immunized by the standard protocol with *T. spiralis*. TDL obtained from these doubly immunized subjects were separated by affinity chromatography into fractions that were enriched in either T cells or B cells. The two fractions, and an equal number of unseparated TDL, were incubated *in vitro* at 37° for 10 h in medium containing 1·0 μg/ml of vinblastine. Parallel cultures lacking vinblastine were maintained at 37° and at 4°. Immediately after incubation, the cells were washed and

2×10^8 were transferred intravenously into individual recipients. All were challenged orally with 700 *T. spiralis* larvae. In addition, the recipients of B cells were injected intravenously with 10^{10} plaque forming units of ϕX.

Under the culture conditions employed there was a substantial reduction in viability among T cells maintained at 37°; however, their capacity to transfer resistance to *T. spiralis* was unimpaired (Table 7). The above finding implies that protective lymphocytes in the T cell fraction divide infrequently or not at all. Vinblastine had relatively little effect on the viability of B cells, although it completely erased their protective power. The action of vinblastine was clearly related to its ability to selectively inhibit dividing lymphocytes. The majority of non-dividing small lymphocytes in the culture were not only viable at the time of cell transfer, but they were functionally active as evidenced by their ability to transfer ϕX memory.

DISCUSSION

Rats can be solidly immunized against *T. spiralis* by a series of 4 methyridine-abbreviated oral infections with isolated muscle larvae (Table 1). Some immunized unchallenged rats harboured a few larvae in their muscles, indicating that the drug did not always eradicate the infection. However, a 99% reduction *was* achieved and this effectively prevented the debilitation associated with the migrating phase of the infection (Gould, 1970).

A single injection of methyridine given to rats 48 h after challenge allows exposure of the animal not only to larval stage antigens, but also to antigens derived from the early adult stage of the parasite (Ali Kahn, 1966; Kozek, 1971). Granule-bound stichocyte antigens of the 1st–4th stage larvae are secreted into the host during the first 36 h of the infection (Despommier, 1974). These larval secretions are immunogenic and are known for their ability to induce a protective response (Campbell, 1955; Despommier & Wostmann, 1968; Mills & Kent, 1965; Despommier & Müller, 1976). Adult secretions also can immunize (Chipman, 1957). However, it is unlikely that antigens associated with the adult stage of *T. spiralis* are responsible for the resistance manifest in methyridine-treated subjects. Thus, immunity comparable to that achieved in the current study is induced when therapeutic doses of methyridine are administered to rats at 24 and 48 h, rather than at 48 h only (Despommier & McGregor, unpublished). Sequential treatment with the drug at 24 h and 48 h eradicates all fourth stage larvae from the gut, and therefore excludes exposure of the host to adult antigens.

Injection of serum taken from rats immunized by our standard methyridine-treatment protocol failed to protect normal recipients against a challenge infection with *T. spiralis*. This finding does not exclude a protective role for antibodies, but it does imply that such antibodies are either absent from serum or they are not present in sufficient concentration to be demonstrated by passive transfer. An entirely different result was reported by Love, Ogilvie & McLaren (1976). They found that serum from rats convalescing from an uninterrupted *T. spiralis* infection can confer substantial immunity on recipient rats. The seeming disparity with our own results could be explained in several ways. However, it is tempting to speculate that animals responding to an uninterrupted infection develop protective antibodies against antigens of the adult parasite.

Whereas the serum of our hyperimmunized rats was devoid of protective activity, resistance *was* transferred adoptively by living cells obtained from the thoracic duct lymph of the same immunized donors. The above finding indicates that the protective cells are lymphocytes, and reveals the route by which they enter the blood.

Subsequent experiments demonstrated that the lymph of specifically immunized subjects contains two populations of antigen-activated lymphocytes. Evidence to support this proposition emerged from experiments using affinity column chromatography (Crum & McGregor, 1976) to fractionate immune TDL. Protective lymphocytes were found in fractions that were enriched in either T cells or B cells. The efficiency of the separation procedure leaves little doubt that the two cell fractions contained distinct populations of antigen-activated lymphocytes. This view is supported by results obtained in earlier experiments (Crum & McGregor, 1976) which demonstrated that the fractionation procedure separates rat TDL into populations that coincide or closely overlap with T and B cells.

Additional evidence that the lymph of *T. spiralis* immune donors contains two populations of antigen-activated lymphocytes was secured in an experiment in which column-fractionated TDL were incubated *in vitro* in the presence of vinblastine. Treatment of

immune T cells had no effect on their capacity to immunize adoptively. In contrast, the drug totally ablated the protection afforded by B cells. Vinblastine was used at a concentration that inhibited dividing lymphocytes selectively. Nondividing B cells were spared, as evidenced by the failure of the drug to inhibit small lymphocytes of the kind which carry immunological memory of ϕX 174.

The results of the current investigation indicate that antigens of *T. spiralis* larvae provoke a protective response that involves at least two classes of activated lymphocytes: T cells which divide infrequently or not at all, and B cells which are delivered to the thoracic duct and hence to the blood while still in active cycle. The mechanisms by which these two cell-types realize their immunological purpose is the subject of a companion report (Despommier, McGregor, Crum & Carter, 1976).

ACKNOWLEDGMENTS

This work was supported by grants AI-08642 and AI-10627 from the National Institute of Allergy and Infectious Diseases (NIAID), D. D. Despommier is the recipient of a Career Development Award from NIAID.

The technical assistance of Ms Pamela Logie, Ms Lorna Aron, Mr Kenneth Roberts and Mr Stephen Langley is gratefully acknowledged.

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