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T-cell co-operation in the mediation of acquired resistance to *Listeria monocytogenes*

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Summary. Monoclonal antibodies were used to select T-cell subsets that mediate delayed-type hypersensitivity (DTH) and acquired cellular resistance (CRI) in rats infected with *Listeria monocytogenes*. The mediators of DTH were identified as W3/25$^+$ OX8$^-$ T cells. The latter comprised a subset distinct from that which could protect recipient rats against a *Listeria* challenge. The protective T cells had a W3/25$^-$ OX8$^+$ phenotype. The T-cell mediators of cellular resistance to infection (TCRI) failed to augment the expression of DTH; however, the mediators of DTH (TDTH) significantly enhanced the protective capacity of TCRI. This property of TDTH correlated with the ability of the cells to promote the focal deployment of TCRI and macrophages at sites of soluble *Listeria* antigen injection in skin, and in peritoneal exudates induced by killed *L. monocytogenes*. These findings illustrate the co-operative interaction of activated T cells in acquired resistance to *L. monocytogenes*, and imply that DTH has a purposeful role in the host defence against infection.

Abbreviations: CRI, cellular resistance to infection; DTH, delayed-type hypersensitivity; FT, *Francisella tularensis*; sFTA, soluble fractions of FT; I-OX8$^+$ T cells, immune OX8$^+$ T cells; LM, *Listeria monocytogenes*; sLMA, soluble fractions of LM; mAB, monoclonal antibody; MHC, major histocompatibility complex; PPD, purified protein derivative of tuberculin; TDL, thoracic duct lymphocytes; N-TDL, normal TDL; T-TDL, T-cell fraction of TDL; TdR, thymidine.

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INTRODUCTION

Immunologists have long debated whether delayed-type hypersensitivity (DTH) is the basis of a cell-mediated defence against intracellular parasites. Proponents of this view have drawn support for their position from parallels between DTH and acquired cellular resistance to infection (CRI). The two phenomena have similar induction kinetics and develop in unison, at least during the early postinfection period (Mackaness, 1967; Mackaness, 1972). Furthermore, DTH and CRI are mediated by T cells (Blanden & Langman, 1971; Lane & Unanue, 1972; North, 1973), and both depend for their full expression upon the co-operative interplay of T cells and monocyte-derived macrophages (Mackaness, 1969; Hahn & Kaufmann, 1981).

The foregoing observations suggest that DTH and CRI are mediated by a single population of antigen-activated T cells, yet numerous examples of dissociation between DTH and CRI have been observed following natural infection or vaccination (Hahn & Kaufmann, 1981). Advocates of the single mediator T-cell hypothesis have argued that such dissociations are more apparent than real, and are related to the sequestration of mediator T cells in lymhoid tissue or the deflection of T cells and macrophages from DTH reaction sites in skin. However, other evidence encourages the belief that sensitivity and resistance are mediated by distinctive T-cell subsets. Thus, differences have been reported in the size, sensitivity to

ionizing irradiation and effective lifespan of T cells which can transfer DTH and CRI adoptively (Lefford & McGregor, 1978). More recently, it has been shown that the cells concerned are restricted by genes that map to either different or overlapping regions of the major histocompatibility complex (MHC) (Cheers & Sandrin, 1983; Jungi *et al.* 1982a). The most compelling evidence for the involvement of distinctive subsets of activated T cells in infection immunity was provided by Orme & Collins (1984). Using the adoptive transfer technique, these investigators demonstrated in mice that sensitivity to the purified protein derivative of tuberculin (PPD) and acquired resistance to tuberculosis are transferred by splenic T cells which can be distinguished phenotypically by monoclonal antibodies (mAb).

The experiments reported here confirm and extend these observations in rats infected with the facultative intracellular bacterium *Listeria monocytogenes* (LM). It will be shown that DTH and CRI are mediated by T cells that have distinctive antigenic profiles. However, the results go further in demonstrating that the cells concerned have different affinities for inflamed tissue, and that the T-cell mediators of DTH (TDTH) co-operate with the T-cell mediators of acquired cellular resistance (TCRI) and macrophages in limiting the growth of LM. These findings give new perspective to the role of DTH in the host defence against infection: they suggest that DTH is the basis of an amplification mechanism whereby TCRI and macrophages are rapidly deployed at sites of microbial invasion.

MATERIALS AND METHODS

Animals

Specific pathogenic-free male and female AO rats were used. The animals were bred at the Baker Institute. T-cell donors were 8–12 weeks old and weighed 150–300 g. Antimicrobial resistance was measured in weanling rats that weighed 60–90 g, while DTH was measured in adults that weighed 200–300 g.

Microorganisms and their 'antigens'

The EGD strain of *Listeria monocytogenes* (LM) and the LVS strain of *Francisella tularensis* (FT) were used. The bacteria were passaged in rats and recovered from infected spleens. LM and FT were grown in trypticase soy broth and T medium (Tresselt & Ward, 1964), respectively. The organisms were stored in liquid nitrogen for up to 6 months. Soluble fractions of LM (sLMA) and FT (sFTA) were prepared from organisms grown to late log phase as described elsewhere (Woan & McGregor, 1981).

Immunization of rats

Prospective donors of thoracic duct lymphocytes (TDL) were immunized with approximately 5×10^6 living organisms. The bacteria, in a volume of 0·6 ml of saline, were injected subcutaneously into both footpads, the base of the tail, and over the lower abdomen. The thoracic duct of rats immunized with LM was incannulated 5 days later. Lymph issuing from the fistula was collected for 10–20 hr into heparinized rat Ringer's solution (20 U/ml of heparin) without added antibiotics. TDL from rats immunized with FT were collected over a similar period 6–7 days after infection.

Monoclonal antibodies

The monoclonal antibodies W3/25 and MRC OX8 were used. Both were purchased as ascitic fluids from Accurate Chemicals and Scientific Corporation (Hicksville, NY).

Preparation of T-cell subsets

Thoracic duct lymphocytes (TDL) from several donor rats were pooled. The cells were passaged through nylon wool to remove the majority of B cells (Julius, Simpson & Henzenberg, 1973). The non-adherent T-cell enriched cells (T-TDL) were incubated for 20 min at 4° at a density of 10^8 T-TDL/ml in medium (RPMI-1640) containing 1:100 dilution of mAb. The cells were then washed twice with PBS and incubated for 30 min at room temperature in petri-dishes coated with affinity column-purified goat anti-mouse IgG (a mixture of 10 μg/ml of affinity-purified Ab plus 90 μg/ml of normal goat IgG in PBS). The non-adherent cell fraction was 'panned' again on a second set of Ab-coated dishes to deplete further the population of adherent cells. Non-adherent cells from the second set of plates were poured off and reserved. Thereafter, the plates were washed gently with medium and the few cells in the wash discarded. Adherent cells were released by vigorous pipetting with fresh medium. Nearly all T-TDL applied to the plates were recovered in the adherent and non-adherent fractions. Cell viability exceeded 99%, as judged by trypan blue exclusion. The subset composition of each fraction was determined by staining the cells with a fluoresceinated rabbit anti-mouse heavy and light chain IgG which had been passaged through a rat IgG Sepharose 4B column to remove cross-reactive antibodies. Cells binding the individual mAb were scored visually using a fluorescence microscope or with a fluorescence-acti-

vated cell sorter (FACS IV, Becton-Dickinson, Sunnyvale, CA). Subset-enriched T-TDL fractions which were selected with the W3/25 or OX8 mAb contained less than 4% of reciprocally marked cells.

Radioactive labelling of T lymphoblasts

Lymphoblasts were labelled selectively by incubating subset-enriched T-TDL *in vitro* for 90 min in medium containing 5% fetal bovine serum and either [^{14}C]thymidine ([^{14}C]TdR, 60 mCi/mmol) or [^{3}H]thymidine ([^{3}H]TdR, 5 Ci/mmol) at a final concentration of 0·5 μCi/ml (McGregor & Logie, 1973).

Protection assay

Resistance to LM was measured in adoptively immunized rats as described elsewhere (McGregor & Logie, 1973). In brief, recipients of T-TDL or subset-enriched fractions thereof were challenged with approximately 3×10^6 LM. The organisms were injected i.v. 1 hr prior to cell transfer. Challenged rats were killed 63–66 hr after infection. The spleens of individual animals were homogenized in saline and plated on trypticase soy agar for quantification of LM. The level of protection was expressed as the difference between the mean number ($\log_{10}$) of bacteria in the spleens of adoptively immunized animals and the spleens of similarly challenged but unprotected controls.

DTH assays

Delayed type hypersensitivity to the subject organisms was measured at reaction sites in the skin and peritoneal cavity. The technique described by Lefford (1974) was used. Briefly, subject rats were injected subcutaneously with [^{3}H]thymidine (0·5 μCi/g of body weight) to label dividing precursors of macrophages in bone marrow. Twenty-four hours later, 3 μg of sLMA or sFTA in 0·04 ml of saline were injected into the pinna of one ear, while a similar volume of saline was injected intracutaneously into the opposite ear. Peritoneal exudates were induced in the same rats by injecting the animals i.p. with 50 μg of alcohol-killed organisms. The organisms, in 1 ml of saline, were injected through the linea alba to avoid local bleeding. Animals stimulated in this manner were infused with T-TDL or T-subset enriched T-TDL fractions prepared from the lymph of immune donors. The animals were killed 24 hr after stimulation. Uniform samples of tissue were removed from the skin reaction sites with a biopsy punch. Immediately thereafter, the exudates were harvested by flushing the peritoneal cavity with 20 ml of medium containing 2% fetal bovine serum and 1 U of heparin. The tissue samples and exudates from individual rats were separately analysed for cell-associated radioactivity as described elsewhere (McGregor & Logie, 1974).

Statistical methods

Paired groups were compared by Student's *t*-test. When more than one comparison was made, differences between groups were determined by analysis of variance and the studentized range test. Linear regression analysis was performed by the method of least squares (Snedecor & Cochran, 1967).

RESULTS

Mediation of DTH by W3/25$^+$, OX8$^-$ T cells

Monoclonal antibodies were used to prepare T-subset enriched fractions from the lymph of 6-day LM immune rats. Two experiments were performed—one in which W3/25 was employed as the selecting mAb, and another in which OX8 was used. Adherent and non-adherent cell fractions prepared in this way were transferred separately or together into normal recipients in proportion to the representation of the respectively marked T cells in donor lymph (W3/25$^+$ T cells : OX8$^+$ T cells = 2 : 1). The cells were infused 24 hr after the animals had been pulsed with [^{3}H]TdR and immediately after they had been stimulated intracutaneously with sLMA.

Table 1 indicates that DTH was conveyed by donor T cells which expressed the W3/25 marker, but not by cells bearing the determinant recognized by OX8. The reactions manifest in recipients given both subset-enriched fractions were not greater than those in rats infused with W3/25$^+$ OX8$^-$ T cells alone. Since the subset 'purity' of the fractions selected with the OX8 mAb was greater than that prepared with the W3/25 mAb, the former was used in all subsequent experiments.

Mediation of CRI by OX8$^+$ T cells

T-TDL from 6-day LM immune rats were fractionated by panning using OX8 as the selecting mAb. Adherent (I-OX8$^+$) and non-adherent (I-OX8$^-$) cells were transferred separately into groups of recipient rats. All had been challenged intravenously with LM 1 hr prior to cell transfer. Other, similarly challenged, recipients were infused with unfractionated T-TDL from the same panel of donor rats.

Figure 1 shows that the I-OX8$^+$ T cells were more

Table 1. Transfer of DTH to sLMA by W3/25$^+$, OX8$^-$ T cells*

Exp.	Selective mAb	T-cell phenotype	No. cells transferred ($\times 10^7$)	Macrophage-associated counts (c.p.m.)†			
				sLMA site	Saline site	Difference	sLMA/saline
1	W3/25	–	–	18,096±1083	12,006±1295	6090	1·51
		W3/25$^+$	24	25,065±904	10,563±364	14,502	2·37‡
		W3/25$^-$	12	18,745±775	11,516±175	7229	1·62
		W3/25$^+$+W3/25$^-$	24+12	27,838±1631	11,274±909	16,564	2·47‡
2	OX8	–	–	6072±597	4322±688	1750	1·41
		OX8$^+$	11	7550±491	4963±488	2587	1·52
		OX8$^-$	21	17,113±1876	4143±290	12,970	4·13‡
		OX8$^+$+OX8$^-$	11+21	15,295±1920	4686±891	10,609	3·26‡

* LM-immune T-TDL subsets were selected with the W3/25 mAb (Experiment 1) or the OX8 mAb (Experiment 2). The selected cells were infused into normal recipients which had been pulse labelled with [^{3}H]thymidine and stimulated i.c. with sLMA or saline (see text).

† Mean of 5±SE.

‡ Significantly different from control group receiving no cells, $P<0·01$, studentized range test.

potent than unfractionated T-TDL in their capacity to inhibit the growth of LM in the recipients' spleens. The protective power of the OX8$^+$-enriched subset was far greater than the fraction enriched in I-OX8$^-$ T cells. Linear regression analysis revealed that the slopes of dose-response lines between the immunity conveyed by the two subset-enriched fractions were not significantly different ($P>0·05$). This relationship suggests that the low level of activity vested in the I-OX8$^-$ subset was related to the activity of the few OX8$^+$ cells in that fraction. A significantly different dose-response relationship was observed in recipients of the unfractionated LM-immune T-cell population (I-T-TDL) however. The latter finding raises the question of whether I-OX8$^-$ T cells enhance the immunity conveyed by I-OX8$^+$ T cells. The following experiment was undertaken to test this proposition.

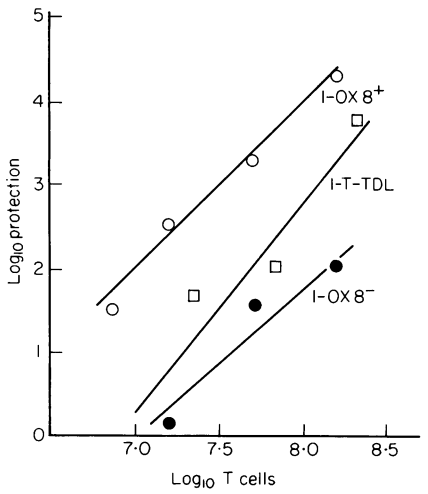

Figure 1. Titration curves showing the immunity conferred on recipient rats by subset-enriched T-TDL or unfractionated T-TDL of 6-day LM-immune donors. The recipients were infused with the specified number of T cells per 100 g body weight. Each point represents the mean difference in number of viable LM in the spleens of five adoptively immunized recipients and five non-immunized controls 66 hr after the animals were challenged i.v. with $3·4\times10^6$ LM.

Augmentation of CRI by LM-immune OX8$^-$ T cells

Three groups of LM-infected rats were infused with a constant number (7×10^7) of I-OX8$^+$ T cells and varying numbers of I-OX8$^-$ cells from the same panel of donor rats. Normal T-TDL were used as 'filler cells' so that each animal received the same number of lymphocytes ($2·1\times10^8$/rat). The make-up of the immune subset-enriched T-cell fractions was determined by FACS analysis and adjusted before injection, so that the individual fractions contained the specified number of I-OX8$^+$ and I-OX8$^-$ cells. Table 2 indicates that substantial protection was afforded by I-OX8$^+$ T cells alone; however, even greater immunity was conveyed when I-OX8$^-$ T cells were also infused.

Localization of T cells in inflammatory foci

Delayed-type hypersensitivity and acquired resistance to LM are mediated in rats by recently activated T cells, many of which are in S phase of the mitotic cycle (McGregor & Logie, 1973). It was of interest, there-

Table 2. Augmentation of CRI by LM-immune OX8$^-$ T cells*

Groups	No. cells transferred ($\times 10^7$) I-OX8$^+$	I-OX8$^-$	N-T-TDL	Viable LM† ($\log_{10}$) Mean ± SE	Protection‡ ($\log_{10}$)
1	7	14	–	1·36 ± 0·15	5·50
2	7	4·7	9·3	2·38 ± 0·20	4·48
3	7	<0·1	14	2·91 ± 0·08	3·95
4	–	–	21	6·86 ± 0·14	–

* LM-immune T-TDL subsets were selected with the OX8 mAb. I-OX8$^+$ T cells were combined with varying numbers of I-OX8$^-$ cells or N-TDL and the mixtures infused into normal recipients. The latter were challenged i.v. with $2·9 \times 10^6$ LM.

† Number of LM in the spleen 63 hr after challenge. Mean of 5 ± SE.

‡ Difference in viable LM when compared with rats infused with N-T-TDL (Group 4).

fore, to determine whether I-OX8$^-$ T lymphoblasts and their I-OX8$^+$ counterparts find their way from the blood into DTH reaction sites. The problem was studied by separately labelling T lymphoblasts in subset-enriched T-TDL fractions prepared from the lymph of LM-immune or FT-immune rats. I-OX8$^-$ T lymphoblasts were labelled with [^{3}H]TdR, while the I-Ox8$^+$ cells from the same donors were labelled with [^{14}C]TdR. The two labelled cell fractions were then recombined in the proportions present in donor lymph and the cell mixture transferred intravenously into similarly immunized recipients. The latter were stimulated intracutaneously with soluble microbial antigens and intraperitoneally with killed organisms.

Two experiments were performed—one with LM-immune rats, and another with rats immunized with FT. The results were similar, insofar as I-OX8$^-$ T lymphoblasts and those expressing the OX8 marker found their way in substantial numbers from the blood into DTH reaction sites in the skin (Fig. 2). In both instances, the exudate-seeking capacity of the I-OX8$^-$ cells was superior to that of T cells bearing the OX8 marker. Stimulation of the recipients by antigens of the organisms with which the animals had been immunized enhanced the extravasation of labelled cells at the injection sites.

Figure 3 shows that these same relationships prevailed in the inflamed peritoneal cavity. In LM-immune rats and rats immunized with FT, there was a preferential movement of labelled I-OX8$^-$ T lymphoblasts from the blood into the exudates. The traffic of both T-cell subsets into the peritoneal cavity was also significantly greater in animals that were stimulated by organisms against which they had been specifically immunized ($P < 0·025$, Student's t-test).

Other experiments (not shown) in which LM-immune T-cell subsets were selected with the W3/25 mAb and labelled with the alternative form of TdR gave similar results. In these experiments, as in the experiments shown in Figs 2 and 3, labelled I-OX8$^-$ T cells showed a preference for inflammatory foci, and the cells extravasated in especially large numbers into reaction sites induced by LM in the skin and peritoneal cavity of specifically immunized rats.

I-OX8$^-$ T cells promote the focal deployment of I-OX8$^+$ T cells

In order to ascertain the influence of I-OX8$^-$ T cells on the exudate-seeking capacity of I-OX8$^+$ T lymphoblasts, T-TDL from LM-immune donors were first divided into subsets using the OX8 mAb as the selecting reagent. Lymphoblasts in the OX8$^+$ cell fraction were then labelled with [^{14}C]TdR. The labelled cells were recombined with either unlabelled I-OX8$^-$ T cells or an equal number of unlabelled TDL from normal, non-immunized rats (N-TDL). The cell mixtures, or an equal number of N-TDL, were infused into normal, non-immunized recipients. The latter had been pulsed with [^{3}H]TdR 24 hr before cell transfer to label blood monocyte precursors (Lefford, 1974; Volkman, 1966). All were stimulated intracutaneously with sLMA and intraperitoneally with killed LM as in the preceding experiments.

Figure 4a shows that I-OX8$^+$ donor T lymphoblasts found their way in substantial numbers into cutaneous DTH reaction sites. Their extravasation in this loca-

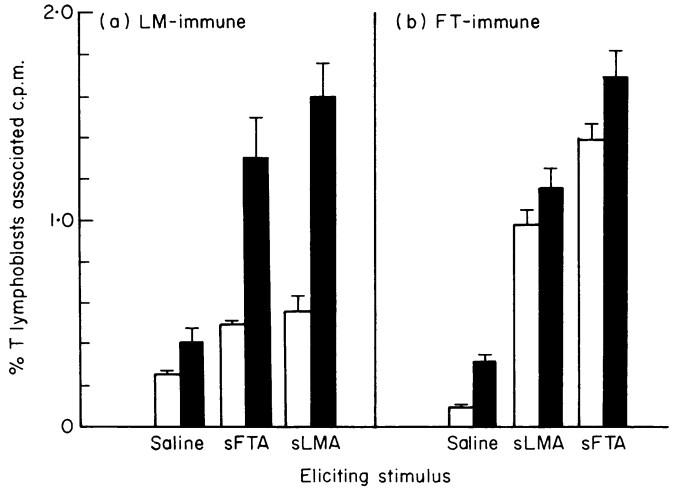

Figure 2. Localization of radioactively labelled T lymphoblasts in DTH reaction sites in skin. I-OX8$^+$ cells (□) and I-OX8$^-$ cells (■) were separately labelled with [^{14}C]thymidine and [^{3}H]thymidine, respectively. The cells were then mixed in proportion to their representation in donor lymph and infused into similarly immunized recipients. Labelled I-OX8$^-$ cells showed a preference to localize in saline injection sites and in inflammatory exudates induced by either sLMA or sFTA. Both I-OX8$^-$ T cells and I-OX8$^+$ T cells extravasated in especially large numbers into reaction sites induced by antigens to which the animals had been specifically sensitized. Mean of $5 \pm$ SE.

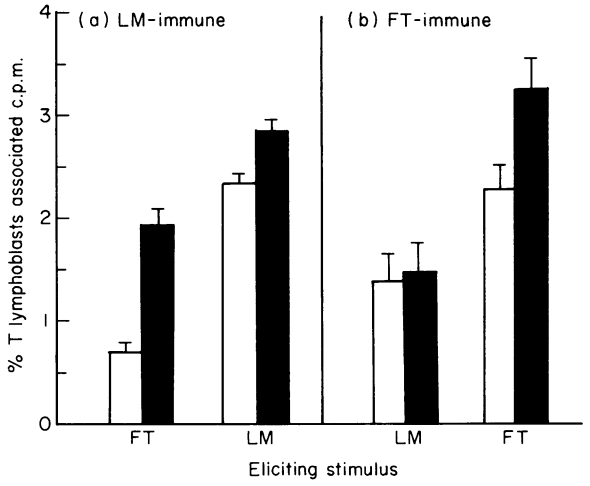

Figure 3. Localization of radioactively labelled T lymphoblasts in peritoneal exudates borne by the same animal shown in Fig. 2. Labelled I-OX8$^-$ cells (■) outperformed I-OX8$^+$ cells (□) in their exudate-seeking capacity. The extravasation of both subsets was significantly enhanced in specifically sensitized subjects. Mean of $5 \pm$ SE.

tion was enhanced in recipient rats which were infused with unlabelled I-OX8$^-$ T cells from the same LM-immune donors. I-OX8$^+$ T cells alone had no obvious attractive influence on [^{3}H]TdR-labelled host cells, the majority of which were macrophages (Fig. 4b). However, the presence of I-OX8$^-$ T cells in the donor inoculum greatly enhanced the local influx of labelled macrophages ($P < 0{\cdot}001$, studentized range test).

Essentially the same pattern of cell localization was observed in the inflamed peritoneal cavity. Here, as in DTH reaction sites in skin, unlabelled I-OX8$^-$ T cells encouraged the influx of [^{14}C]-labelled I-OX8$^+$ donor T lymphoblasts (Fig. 5a) and [^{3}H]-labelled host macrophages (Fig. 5b) ($P < 0{\cdot}005$, studentized range test).

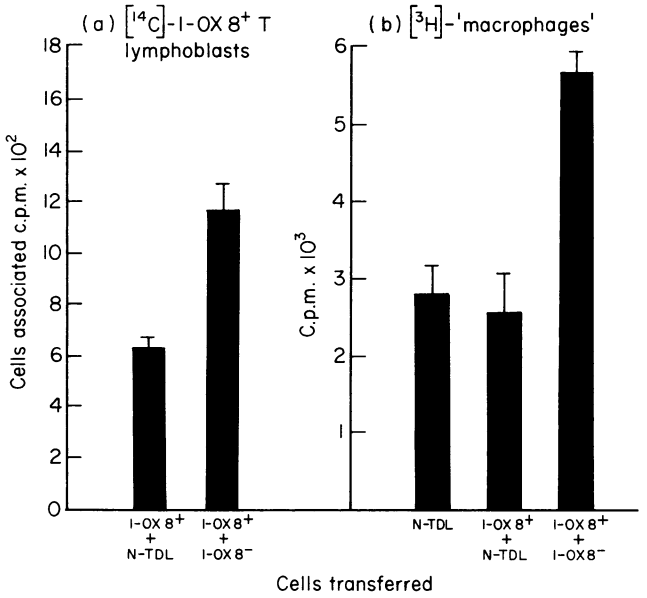

Figure 4. Localization of radioactively labelled donor T lymphoblasts and labelled host 'macrophages', in DTH reaction sites induced in the skin of adoptively sensitized rats. Donor T lymphoblasts and host macrophages were labelled with [$_{14}$C]thymidine and [$_3$H]thymidine, respectively (see text). Recipient rats were infused with a mixture of unlabelled TDL from normal non-immunized donors or a mixture of labelled OX8$^+$ T cells from LM-immune rats and either unlabelled N-TDL or unlabelled I-OX8$^-$ T cells from the same panel of LM-immune donors. I-OX8$^-$ cells significantly enhanced the local extravasation of labelled I-OX8$^+$ lymphoblasts in the reaction sites. I-OX8$^+$ cells had no discernable effect on the localization of labelled macrophages; however, the accumulation of macrophages at reaction sites was greatly enhanced by the addition of I-OX8$^-$ cells to the donor inoculum. Mean of 5 ± SE.

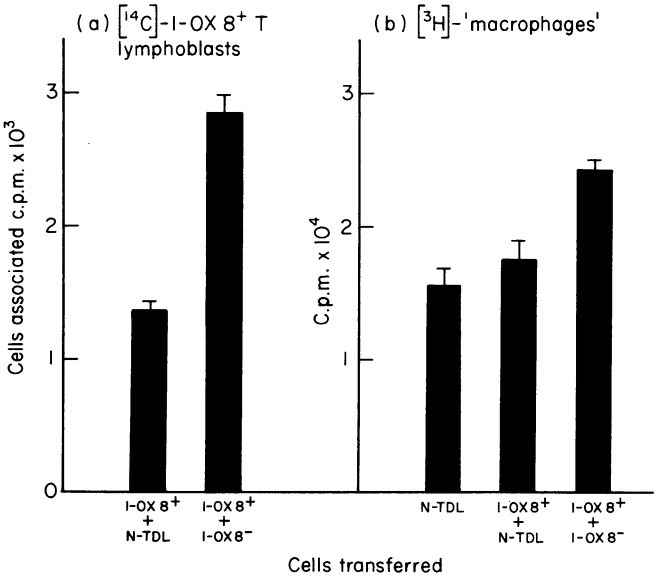

Figure 5. Localization of [^{14}C]-labelled donor I-OX8$^+$ T lymphoblasts and [^{3}H]-labelled host 'macrophages' in LM-induced exudates borne by the same animals shown in Fig. 4. Unlabelled I-OX8$^-$ T cells promoted the accumulation of both (a) labelled I-OX8$^+$ T lymphoblasts and (b) [^{3}H]-labelled macrophages in the exudates. Mean of 5 ± SE.

DISCUSSION

Two major findings emerged from this investigation. The first was that DTH and acquired resistance to LM are mediated in rats by T cells that have distinctive characteristics and different functions. The second finding was that TDTH and TCRI co-operate in the host defence against infection.

The T-cell mediators of DTH and CRI were identified by their capacity to bind mAb under conditions that promoted attachment of the cells to antibody-coated petri-dishes. The two mAbs used for this purpose, W3/25 and OX8, recognize determinants that are reciprocally expressed on rat peripheral T cells (Brideau *et al.*, 1980). Although T-cell fractions enriched in the designated subsets could be selected with either reagent, preparations of the highest 'purity' were prepared with the OX8 mAb. The superior performance of this antibody is probably related to the larger number of anti-OX8 molecules that bind to rat T cells under saturating conditions (Mason *et al.*, 1980). However that may be, subset-enriched T-cell fractions were prepared with either mAb in the numbers required to assay their performance in recipient rats, and to trace the migration of the cells from the blood into DTH reaction sites.

Two additional points merit comment in regard to the use of the specified mAbs as selecting reagents. Firstly, binding of the antibody to rat T cells failed to impair the mediator function of the cells or their capacity to localize in inflammatory foci. The behaviour of positively selected (mAb-coated) cells was not obviously different from negatively selected (uncoated) T cells. This finding implies that binding of the mAb to the cell surface did not facilitate phagocytosis or lysis of the cells by complement, or their capacity to recognize inflamed vascular endothelium.

The ability of LM to engage TDTH that have a W3/25$^+$, OX8$^-$ phenotype accords with earlier reports by Crum (1983) and by Ernst & Lubaroff (1984). These investigators showed that T-cell mediators of DTH to tuberculin PPD express the W3/25 marker at sufficient density to allow fractionation of the cells on affinity columns or by panning. Similar studies in mice have demonstrated that the T-cell mediators to non-replicating antigens are, for the most part, Lyt 1$^+$2$^-$ T cells (Leung & Ada, 1980). Antibodies to T cells that fail to recognize the Lyt 2 marker have a predominantly helper/inducer function and, thus, coincide or closely overlap the subset specified by the W3/25 mAb in rats. Similar experiments using antibodies to Lyt carry less conviction because the Lyt 1 marker is expressed on most murine T cells (Ledbetter *et al.*, 1980). Nevertheless, Orme & Collins (1984) demonstrated that an mAb to Lyt 1 can distinguish TDTH and TCRI obtained from mice immunized with living BCG. Treatment of the cells with this mAb in the presence of anti-rat serum and rabbit complement blocked their capacity to transfer DTH to tuberculin PPD, but did not impede the cells' ability to protect recipient mice against a challenge infection with virulent tubercle baccili.

Kaufmann, Simon & Hahn (1979) performed similar experiments in *Listeria*-infected mice, and concluded that TCRI have an Lyt 123$^+$ phenotype. The same investigators showed that a cloned Lyt 1$^+$2$^-$ T-cell line could also protect recipient mice against LM, but only when the cells were injected locally into centers of infection (Kaufmann & Hahn, 1982). This finding was taken as evidence to suggest that the cloned cells had lost their exudate-seeking capacity. In a subsequent report, Naher, Sperling & Hahn (1984) suggested that LM-protective T cells belong to an activated T-cell line, members of which differ in respect to turnover and expression of Lyt markers. They concluded that immature cells have a Lyt 123 phenotype and a propensity to localize in inflammatory foci. The more differentiated Lyt 1$^+$2$^-$ progeny of the early phase cells no longer recognize inflamed vascular endothelium, although they are the ultimate mediators of CRI. However, an equally plausible explanation is that the cloned cells analysed by Kaufmann and his associates were altered in culture in ways that occur infrequently or not at all in the intact animal. A change of this sort might involve the loss of surface antigens (including Lyt 2), and the ability of the cells to engage inflamed endothelium. This type of change might occur without impeding the proliferative potential of the cells or their capacity to release lymphokines when stimulated by LM antigens.

However this may be, the finding that TDTH and TCRI have distinctive phenotypic profiles implies that viable LM excite the formation of at least two populations of mediator T cells. This conclusion was arrived at earlier by Jungi *et al.* (1982b) who used the tool of restricted recognition to demonstrate diversity amongst T cells that transfer DTH and CRI to LM in rats. Compatibility in the RT1 B region between actively immunized (infected) donors and normal recipients was found to be both necessary and sufficient for the transfer of DTH to either sLMA or killed LM. By comparison, RT1 A region compatibility

allowed the transfer of higher levels of resistance than did transfers between donor–recipient pairs that shared the same B region.

While the foregoing observations favour the view that DTH and CRI are mediated by functionally distinct T-cell subsets, they are subject to the reservation that the hypersensitivity elicited by living organisms may involve the activity of different or (more likely) additional mediator T cells. There is evidence to support this view. In both rats and mice, the phenotype of TDTH and the restriction imposed on these cells is influenced by the nature of the immunizing and eliciting stimuli (Leung & Ada, 1980; Jungi *et al.*, 1982a). The implication is that DTH, as conventionally measured by the cutaneous reaction to soluble antigens, may be an inappropriate model of events in centres of infection.

The T-cell mediators of DTH and CRI in *Listeria*-infected rats are, for the most part, recently activated T cells that are in S phase of the mitotic cycle (McGregor & Logie, 1973). Such T lymphoblasts are generated in regionally stimulated lymphoid tissue, circulate briefly, and leave the blood to infiltrate tissues throughout the body (Jungi & Jungi, 1981). While activated T cells have a limited capacity to extravasate spontaneously, they do so in large numbers in tissues that are the seat of inflammation (McGregor & Logie, 1974). In the current investigation, the tissue tropism of T lymphoblasts was revealed as a property of cells bearing either the W3/25 or OX8 marker. The former showed a preference for both normal and inflamed tissues, but T lymphoblasts of both phenotypes extravasated in especially large numbers into exudates induced by soluble microbial antigens or killed bacteria against which the animal had been specifically immunized. This relationship was observed in both actively and adoptively immunized subjects.

The foregoing observations assume significance in the light of an earlier report by Jungi (1981) who showed that the TDTH of LM-immune rats have a shorter circulating lifespan than *Listeria*-protective T cells, and that the protective power of cells in the peritoneal cavity increases with time relative to the capacity of cells from that compartment to transfer DTH. Evidently, TDTH reach the extravascular spaces more rapidly than do TCRI, but either die sooner or redistribute themselves more rapidly than their protective counterparts.

The affinity of TDTH for inflamed tissues provides a plausible explanation for the capacity of these recently activated T cells to augment the protective function of TCRI. The T-cell mediators of DTH significantly enhance the local deployment of TCRI in reaction sites induced by sLMA or killed LM, and presumably also in *Listeria*-infected tissues. It should be noted in this connection that, while TDTH have little if any protective value in themselves, they caused an exuberant influx of monocyte-derived macrophages into DTH reaction sites. If monocytes are similarly guided into sites of *Listeria* implantation, it would seem that the mere deployment of the cells in tissues cannot account for their protective function. Evidently, macrophages must be functionally modified to kill LM. The present investigation did not address this issue directly. Nevertheless, it was shown that the TCRI of specifically immunized rats had significant protective value against LM, although they were unable to promote an exuberant influx of macrophages into LM reaction sites (Figs 4b and 5b). Taken at face value, these observations suggest that TDTH and TCRI are co-operative participants in a cell-mediated defence against LM.

How do LM-activated T cells co-operate in the host's defence against infection? The data reported here indicate that TDTH have a focusing influence on TCRI and macrophages which encourages the congregation of these cell protagonists at sites of microbial invasion. Tissue-positioned TCRI would be strategically placed to enhance macrophage microbicidal function. This can be said without prejudice, to the view that TCRI exert their influence on macrophages through soluble mediators or by cell contact. The latter possibility cannot be dismissed when it is remembered that LM-immune T cells with the phenotypic characteristics of TDTH can promote the terminal differentiation of LM-dependent cytotoxic T cells (Woan, McGregor & Goldschneider, 1981). The latter express the OX8 marker and share with TCRI the capacity to adhere selectively to syngeneic fibroblasts (Chen-Woan & McGregor, 1984). Although TCRI are contained within this cytotoxic subset, it is not yet known whether their lytic potential is related to their capacity to modify macrophage microbicidal function.

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