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Antigen transport

I. DEMONSTRATION AND CHARACTERIZATION OF CELLS LADEN WITH ANTIGEN IN THORACIC DUCT LYMPH AND BLOOD

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Summary. Thoracic duct lymph (TDL) of non-immune rats and mice was examined for the presence of antigen-carrying cells immediately following a single injection of ^{125}I-labelled or fluorescence-labelled serum protein antigens. Small numbers of cells laden with antigen ($\sim$1/2000 to 1/5000) were identified in TDL and blood by autoradiography or fluorescence microscopy. The antigen-laden (Ag-L) cells resembled macrophages in that a large number adhered to plastic, they phagocytosed bacteria or a particulate dye, were non-specific esterase positive, radioresistant and could take up more than one antigen at one time *in vivo*. Surface phenotyping using monoclonal antibodies against rat cell markers established that Ag-L cells did not express Ia determinants.

The results suggest the existence of a subpopulation of macrophage-related cells that may be involved in the transport of antigen and in stimulation of antibody responses.

Abbreviations: Ag-L cells, antigen-laden cells; TDL, thoracic duct lymph; HSA, human serum albumin; DAB, Dulbecco's phosphate-buffered saline containing 0·1 g calcium chloride and magnesium chloride per litre; BSA, bovine serum albumin; HGG, human gamma globulin; BGG, bovine gamma globulin; FITC, fluorescein isothiocyanate; TRITC, tetraethyl rhodamine isothiocyanate; a.p., alum precipitated.

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INTRODUCTION

The handling of antigen—its distribution, elimination and sequestration—is an important factor in the stimulation and regulation of the immune response. Much is known of the localization and retention of antigen in the spleen and lymph nodes (reviewed by Humphrey, 1982) but with few exceptions (Silberberg-Sinakin, Thorbecke, Baer, Rosenthal & Berezowsky, 1976; Soeberg, Sumerska, Binns & Balfour, 1978) the concept that antigen may be carried to lymphoid tissue by mobile cells has received little experimental support.

In a previous publication (Bell, 1979) we reported the early appearance in rat thoracic duct lymph (TDL) of cells that were apparently transporting antigen following a single injection of human serum albumin (HSA). It was established that cells in TDL, carrying nanogram quantities of HSA, were highly immunogenic *in vivo* in that they stimulated adoptively transferred memory cells to synthesize augmented levels of specific antibody (Bell, 1979). This unexpected finding led to further investigation of lymph for more direct evidence of such antigen-laden cells (Ag-L cells).

The present report provides additional evidence for antigen bearing cells in TDL and describes the macrophage-like properties of these cells, their frequency in lymph, their migration to the blood stream and their surface phenotype. A preliminary account of these studies has been published elsewhere (Bell, 1982). On the basis of these studies the Ag-L cell is compared with other antigen-presenting accessory cells that have been described.

MATERIALS AND METHODS

Animals

Rats from the PVG, AO, DA, BN and PVG.RT1^u strains were obtained from the inbred colonies at Manchester University Medical School. BALB/c mice were from an SPF colony at ICI (Alderley Park).

Irradiation

Rats were irradiated with 500–800 rad from a horizontally directed linear accelerator.

Thoracic duct cannulation

TDL was collected via a polyethylene cannula inserted through a fistula in the duct and secured by a silk suture encompassing the duct and drawn tightly around the cannula (Ford, 1978). Mouse TDL was kindly provided by Miss Jenny Holmes using the Boak & Woodruff (1965) technique.

Cells

TDL was collected on ice into Dulbecco's phosphate-buffered saline containing A + B mineral salts (DAB; Oxoid Ltd) and 20 u./ml heparin. The cells were washed extensively by centrifugation (3–4 cycles) and finally centrifuged through an underlying layer of serum (foetal calf or rat) to remove traces of antigen.

Peripheral blood lymphocytes

Rats were exsanguinated with a heparinized syringe and needle inserted into the lower part of the aorta. Blood from individual rats was diluted three-fold with DAB containing 1 u./ml heparin, divided between two 6 ml Ficoll/Hypaque gradients (63·7 parts 14% Ficoll: 20 parts 45% Hypaque) and centrifuged at 1500 *g* at the interface for 30 min at 15°. The cells at the interface were collected, washed twice with DAB, centrifuged through foetal calf serum and smeared on slides for autoradiography or fluorescence microscopy.

Cell cultures

Thoracic duct cells from antigen injected donors were washed thoroughly, resuspended to 10^8/ml in RPMI-HEPES plus 10% foetal calf serum. Five millilitres were transferred to plastic culture flasks (Falcon) and incubated for 3 hr at 37°. Non-adherent cells were removed by gentle agitation and replaced by fresh culture medium and the flask was incubated overnight. Non-adherent cells were again removed, the medium replaced and the cultures incubated with 100 μl of 0·5% Berlin Blue. Two hours later the cells were fixed *in situ* for 30 min by adding several drops of 25% glutaraldehyde to the flask. After washing, the bottom of the flask was cut free and prepared in the usual way for autoradiography.

Antigens

Bovine serum albumin, human gamma globulin, bovine gamma globulin (BSA, HGG, BGG), ferritin (all from Sigma Ltd) and HSA were conjugated with fluorescein isothiocyanate (FITC) isomer I (BBL, Becton Dickinson & Co.) or tetra ethyl rhodamine isothiocyanate (TRITC) isomer R cryst. (Nordic) by the method described in Hudson & Hay (1976). HSA was iodinated with carrier-free ^{125}I (Amersham) by the chloramine T method of Hunter & Greenwood (1962). Conjugated, iodinated or unlabelled proteins were alum precipitated (a.p.) with $AlK(SO_4)_2$ by the method in Williams & Chase (1967).

Antisera

Rabbit antiserum against mouse IgG was initially absorbed against rat IgG-coupled Sepharose 4B (Pharmacia) and subsequently immunopurified by elution with 0·1 M propionic acid from mouse IgG-Sepharose 4B after the method of Mason & Gallico (1978). The immunopurified antibody was conjugated with FITC for use as an indirect stain for mouse monoclonal antibodies. The following monoclonal antibodies (the kind gift of Drs A. F. Williams, W. R. McMaster and D. W. Mason, Oxford) were used to type rat cell surface antigens: W3/13, W3/25, OX3, OX4 (Williams, Galfre & Milstein, 1977; McMaster & Williams, 1979). Surface Ig was detected with a fluorescent (FITC or TRITC), immunopurified, sheep anti-F(ab′)$_2$ of rat IgG.

Fluorescence staining

All primary and secondary antisera used for staining Ag-L cells were ultracentifuged immediately before use at approximately 80,000 *g* for 45 min to remove

complexes, a procedure that abolished background staining and was essential to prevent non-specific uptake by Ag-L cells. Cells from TDL were washed in cold DAB containing 5% calf serum, 0·02 M sodium azide (DAB-5-Az) using an automatic cell washing system (Sorvall) and held on ice at all incubation stages. A washed cell pellet ($2{\cdot}5 \times 10^6$ cells) was resuspended in 20–50 μl of diluted primary antiserum (control serum or monoclonal diluted with DAB-5-Az) incubated on ice for 1 hr, washed twice and stained with 20–50 μl of fluorescent antiserum (FITC-rabbit anti-mouse IgG) for another hour. The cells were washed twice, resuspended in 20 μl of foetal calf serum and 5 μl drops smeared onto slides. These were fixed in methanol and mounted in buffered glycerol. The smears were examined with a Leitz Ortholux II microscope ($\times$ 100 oil immersion objective) under phase contrast, and by epi-illumination fluorescence using the Ploem interchangeable wavelength filters for FITC and TRITC.

Autoradiography

Slides were dipped in Ilford 65 emulsion, exposed for up to 12 weeks at 4°, developed and stained through the emulsion with methyl-green pyronin.

Antibody assay

Serum antibody levels were determined by a radioactive-antigen binding Farr assay as previously described (Bell & Shand, 1973).

Non-specific esterase

Cell smears were fixed in cold calcium formol or cold formol sucrose for 2 or 5 min, respectively (Evenson-Pearse, 1968), incubated for 20 min with α-naphthyl acetate and fast-blue RR salt and mounted in buffered glycerol for microscopic examination under both fluorescent and bright field illumination. Other smears were counterstained with neutral haematoxylin (Evenson-Pearse, 1968).

RESULTS

Cells from antigen-injected donors were examined directly using radiolabelled or fluorescent antigens for evidence that cells in TDL might carry antigen. Following thoracic duct cannulation rats were injected intraperitoneally (i.p.) with various preparations of antigens, and consecutive hourly samples of TDL were collected in heparinized DAB using a fraction collector. Selected or pooled samples were washed, prepared for autoradiography or fluorescence microscopy and appropriately examined. Table 1 summarizes the results of four representative experiments in which three different antigens were tested, both in the soluble and alum precipitated form. Cells laden with antigen were identified in TDL as early as 3 hr after injection and were continuously present for up to 48 hr later. Approximately 75% of the positive cells identified autoradiographically were heavily labelled (> ten grains) with lightly labelled cells (six to ten grains) comprising the remainder. The frequency of Ag-L cells fluctuated with time and varied between individual experiments. The very large number of Ag-L cells recovered during the 18 hr interval (2570 per 10^6) has no obvious explanation. This high frequency was also reflected by a corresponding increase in the ^{125}I c.p.m. in the washed cell pellet (data not shown). Note also the relatively small number of labelled cells in the preceeding 15 hr interval, hence suggesting a period of shutdown followed by a period of compensatory release. When summated over the entire collection period, the frequency of labelled cells consistently ranged between 1/3000 and 1/5000 between different experiments. The total number of Ag-L cells appearing in TDL was determined in two of the experiments and indicates that approximately 5000–6000 Ag-L cells were mobilized into the thoracic duct each hour (Fig. 1).

Cells in the thoracic duct are almost entirely lymphocytes. Morphologically the cells carrying radiolabelled antigen (Fig. 2) were indistinguishable from large lymphocytes. But from the following studies it was clear that Ag-L cells belonged rather to the macrophage/monocyte series.

Irradiated (800 rad) and non-irradiated, thoracic duct-cannulated rats were injected i.p. with [^{125}I]-apHSA. Lymph recovered during the second overnight collection was cultured *in vitro* in order to allow the attachment of adherent cells and pulsed the next day with Berlin Blue (see Materials and Methods). Autoradiographic examination of the flasks revealed large adherent cells containing radioactive antigen and particles of Berlin Blue, a marker of phagocytosis (Table 2). Approximately half of the cells laden with radioactive antigen *in vivo* were demonstrably phagocytic *in vitro* (^{125}I positive and Berlin Blue positive). There were also cells in TDL that did not carry the radiolabelled antigen but were phagocytic in culture. Irradiation severely depleted the lymphocyte population in TDL but did not abolish the

Table 1. Number of Ag-L cells per million in TDL determined by autoradiography or fluorescent microscopy

Hours after injection of antigen	[^{125}I]-apHSA‡	[^{125}I]-sHSA‡	TRITC-apBSA‡	TRITC-apBGG‡
3		750		
6		350		
8	29		*	*
9		540		
10	48			
11	89			
12	†	360	*	*
15		60		
16			*	286
18		2570		
20			600	400
21	†	250		
24	48	440	364	41
26	282			
27		325		
28	216		194	200
30	194	145		
32	263			
33		120	276	66
35	443			
36		70		
38	417		120	37
39		15		
41	226			
43			310	
44	175			
48			381	
Mean frequency of Ag-L cells	1/3810	1/4084	1/3223	1/5277

* Samples clotted during washing.

† Thoracic duct drainage ceased during this period. Restarted following removal of clots from cannula.

‡ [^{125}I]-apHSA, 10 mg i.p., specific activity 15 μCi/mg; [^{125}I]-sHSA (soluble HSA), 12 mg i.v., specific activity 15·9 μCi/ml; TRITC-apBSA, 10 mg i.p.; TRITC-apBGG, 10 mg i.p.

appearance of Ag-L cells, although the uptake of Berlin Blue in this experiment was considerably reduced.

Additional culture experiments in which TDL donor rats were injected with FITC-apHSA demonstrated that adherent FITC containing cells could ingest TRITC-labelled *Cornyebacterium parvum*. Cells from the TDL of an FITC-apHSA-injected donor were incubated on plastic petri dishes for either 1 or 2 hr. This procedure removed either 57% or 65% respectively of the Ag-L cells but it was not possible to deplete TDL entirely of AG-L cells by this technique.

Ag-L cells were identified in TDL following injection of HSA, BSA, HGG, BGG or ferritin using either alum precipitated or soluble forms of these antigens. The uptake of more than one antigen by Ag-L cells was demonstrated by injecting TDL donor rats simultaneously with FITC-apHSA and TRITC-apBGG. Examination of TDL showed that 75% of all cells carrying antigen were double-labelled suggesting that uptake *in vivo* was not selective.

The staining of Ag-L cells for non-specific esterase was tested in rat TDL by combining fluorescent microscopy with histochemistry. (The esterase

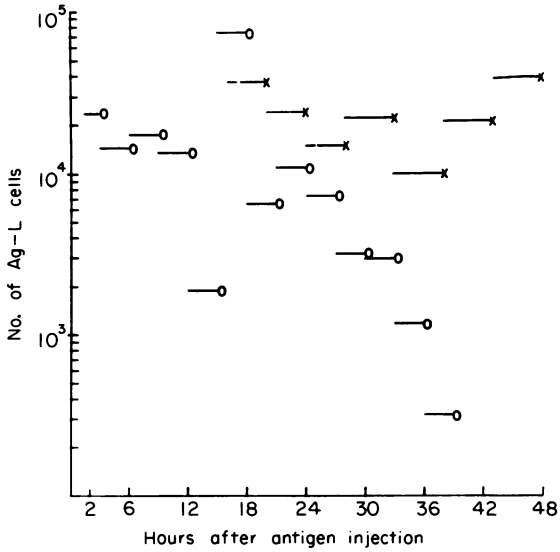

Figure 1. Total number of Ag-L cells in TDL per 3 hr (0) or 4·5 hr (X) interval following the injection of ^{125}I-sHSA (0) or TRITC-apBSA (X). Horizontal bars indicate the collection period.

Table 2. Many Ag-L cells are adherent and phagocytic

	Donor*			
	Non-irradiated		Irradiated	
[^{125}I]-apHSA	+	—	+	—
Berlin Blue				
+	23	29	10	0
—	25	23	56	34

* Donors were injected i.p. after thoracic duct cannulation with 10 mg [^{125}I]-apHSA, specific activity 12·5 μCi/mg.

Figures indicate percentage of adherent cells labelled *in vivo* by radioactive antigen, or *in vitro* by uptake of Berlin Blue.

procedure diminished but did not abolish the fluorescence of labelled antigens.) All the cells in TDL carrying fluorescent antigen were also weakly positive for non-specific esterase (Table 3) but not all esterase-positive cells were laden with antigen.

In order to gain some indication of whether our observations represented a general phenomenon, TDL cells were examined for another species. Six mice bearing indwelling cannulae were injected i.p. with [^{125}I]-apHSA. Cells from the overnight collection of TDL were prepared in the usual way for autoradiography. Three mice produced no results for technical reasons (death, clotting and high background on the autoradiographs). The results of the remaining animals are given in Table 4, demonstrating the presence of radiolabelled cells in the TDL of mice.

The microscopic and *in vitro* studies suggested that Ag-L cells were radioresistant (Table 2). As a confirmation of this, sublethally irradiated donors were injected with a fluorescein-labelled antigen at different times after irradiation with 500–800 rad. The frequency of labelled cells in the TDL approached 1% during the first overnight collection and in several rats increased to 5% after 3 days of continuous drainage. In order to assess their *in vivo* immunogenic properties, Ag-L cells from irradiated donors were tested in an adoptive secondary response. HSA primed thoracic duct lymphocytes (memory cells) were transferred into irradiated recipients and challenged with soluble HSA

Table 3. Ag-L cells in TDL are non-specific esterase positive

TDL donors injected with*	Thoracic duct cells (%) that were†				Cells esterase positive (%)	Cells with fluorescent antigen (%)	Fluorescent cells also esterase positive (%)
	Lymphocytes		Macrophages	Neutrophils			
	Small	Large					
FITC-apHSA	92·1	6·1	0·9	0·9	1·28	0·077	100
TRITC-apBGG	90·5	7·2	1·5	0·8	0·98	0·054	100

* Ten milligrams injected i.p. following thoracic duct cannulation.

† Smears stained with Wright's stain.

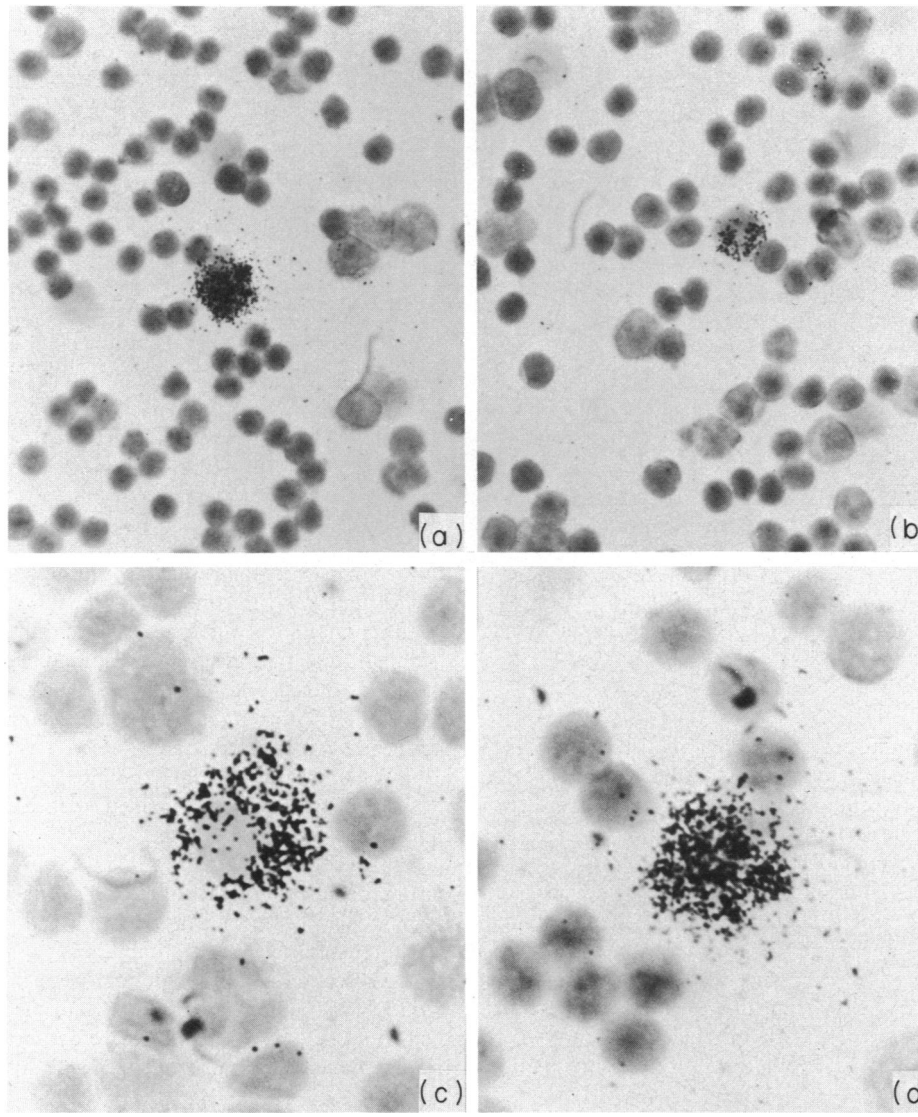

Figure 2. Radiolabelled cells in thoracic duct lymph following the acute injection of ^{125}I-apHSA. [Magnification: (a) and (b) ×600; (c) and (d) ×1120.]

or given Ag-L cells from irradiated or non-irradiated donors in lieu of free antigen (Table 5). The injection of Ag-L cells from irradiated donors stimulated antibody synthesis (Group 4) which was three-fold greater than the antigen-injected control group (Group 1). The results demonstrated that the immunogenic capacity of irradiated Ag-L cells remained intact.

In summary, Ag-L cells in TDL were shown to be large, adherent, phagocytic, esterase positive and radioresistant cells: properties characteristic of macrophages.

Macrophages occur only rarely in TDL and at least one investigator has suggested that their appearance in efferent lymph could be the result of the local inflammatory response initiated by the cannulation procedure (Roser, 1970). Although it is difficult to test this proposition directly, it is possible to examine the

Table 4. Autoradiographic demonstration of Ag-L cells in the thoracic duct lymph of mice acutely injected with [^{125}I]-apHSA*

Mouse no.	No. of cells scanned	No. of radio-labelled cells	Frequency of labelled cells	As a % of total
1	14,800	15	1/987	0·101
2	10,500	8	1/1312	0·076
3	10,300	7	1/1471	0·068

* Mice were injected i.p. with 1 mg [^{125}I]-apHSA after the cannulation procedure.

blood, since cells entering the thoracic duct in the intact animal will empty within seconds into the venous circulation. If the Ag-L cells detected in TDL represented a true physiological migration, they should normally be present in blood.

Six rats were injected with [^{125}I]-apHSA and two were exsanguinated from the aorta on each of days 1, 2 and 3. Ficoll/Hypaque separated white blood cells were prepared for autoradiography. Radiolabelled cells were identified in all six animals at a frequency of approximately 1/3000 (Table 6). It was estimated that 5000–15,000 Ag-L cells were present in blood at the instant of sampling.

The surface phenotype of Ag-L cells was determined by staining cells carrying antigen with mouse monoclonal antisera known to identify subclasses of rat lymphocytes. A double fluorochrome system was used in which donors were injected with TRITC-apBSA to mark Ag-L cells and the cells in TDL were incubated with monoclonal reagent and counterstained with FITC anti-mouse IgG. Four different inbred strains of rats were examined (PVG, AO, DA and BN) at 2–7 days or 6–8 weeks after 500–650 rad. The irradiation effectively reduced the number of lymphocytes thereby enriching the TDL for AG-L cells. The results are summarized in Table 7. The W3/13 monoclonal stains rat peripheral T cells, polymorphonuclear leucocytes and granulocytes, but not macrophages (Williams *et al.*, 1977; Barclay, 1981). Contrary to expectations, a majority of the Ag-L cells were W3/13 positive, although some variation in percentage staining was observed between individual animals. A portion of the W3/13+ cells were judged to be neutrophils under phase contrast but the number was

Table 5. Ag-L cells from irradiated donors augment the adoptive secondary response

Group*	Primed TDL†		Additional TDL‡	Challenge§	Day 21 ABC¶ (±SE $\log_{10}$ ABC)
1	$10^{7·5}$			10 μg sHSA	19·16 (±0·133)
2	$10^{7·5}$	+	10^8 Ag-TDL	None	12·45 (±0·085)
3	$10^{7·5}$	+	10^7 Ag-TDL	None	6·67 (±0·073)
4	$10^{7·5}$	+	4×10^6 Ag-Xrad TDL	None	60·12 (±0·042)

* Recipients, five or six per group, were irradiated with 750 rad the day of cell transfer.

† TDL collected from donors immunized against HSA 4 and 55 weeks before cannulation.

‡ TDL from antigen injected donors: unirradiated (Ag-TDL) or 750 rad irradiated (Ag-X-rad TDL). Donors were injected i.v. with 100 mg sHSA 1 hr before thoracic duct cannulation plus 4 and 20 hr after.

§ Recipients of primed TDL were injected i.p. with soluble HSA after cell transfer, a standard procedure used to stimulate memory cells (Bell & Shand, 1973).

¶ Antigen binding capacity = μg HSA bound/ml neat serum.

Table 6. The frequency of Ag-L cells in blood

Time after injection* (hr)	Rat no.	WBC ($\times 10^{-6}$) per ml blood	No. labelled† cells per 10^6	No. labelled cells/10 ml blood‡	Mean frequency
24	1	2·35	203	4771	1/3650
	2	1·55	420	6510	
48	3	3·33	300	9990	1/3005
	4	2·28	374	8527	
72	5	3·51	205	7195	1/3380
	6	3·00	526	15,780	

* Six month old male PVG rats injected i.p. with 10 mg [^{125}I]-apHSA, specific activity 12·6 μCi/mg.

† Autoradiographs exposed for 6 weeks; only cells with >10 g were scored; 24,000–103,000 cells scanned for each rat.

‡ Seven–ten millilitres of blood were obtained from each rat.

not sufficient to account for the high percentage of W3/13+ Ag-L cells. The W3/25 marker identifies helper T cells and is found on unstimulated peritoneal macrophages (Barclay, 1981); however, Ag-L cells lacked the W3/25 surface antigen. Further investigation demonstrated that peritoneal macrophages lose the W3/25 marker when stimulated by antigen *in vivo* for 18 hr (unpublished). Ia antigen in the rat was detected by the OX4 monoclonal which reacts with Ia determinants on all rat strains examined to date (McMaster & Williams, 1979). Ag-L cells were almost all negative for Ia. The few exceptions were occasional large, strongly Ia positive cells with abundant cytoplasm and a frilly membrane, morphologically like the 'veiled' cells (Drexhage, Mullink, de Groot, Clarke & Balfour, 1979) or 'dendritic' cells (Steinman, Kaplan, Witmer & Cohn, 1979) but such cells carried only a few specks of TRITC-BSA. Many more of these Ia+ dendritic/veiled cells were encountered in TDL that carried no fluorescent antigen. Similar results were obtained from each of the four strains.

Table 7. The surface phenotype of Ag-L cells in TDL detected by fluorescent microscopy

Description of marker	Monoclonal antibody	No. of observations	Ag-L cells carrying marker (%)*
All T cells, polymorphs granulocytes	W3/13	9	21, 38, 51, 55, 90 91, 96, 96, 98
Helper T cells. peritoneal macrophages	W3/25	10	0, 0, 0, 0, 0, 0, 0, 2, 2, 6
Anti-Ia	OX4	9	0, 2, 2, 2, 2, 2, 4, 6, 13
Control	Normal mouse serum	8	0, 0, 0, 0, 0, 2, 9, 10

* Double labelled. Cells in TDL from TRITC-BSA injected donors were stained with monoclonal antibody and FITC-anti-mouse IgG. Slides were scanned with a one hundred times oil immersion objective for the infrequent rhodamine positive cells and immediately scored for the presence or absence of FITC. The percentages were derived in most cases from >50 rhodamine positive cells.

DISCUSSION

By injecting radiolabelled or fluorescent antigens into donor rats it was possible to identify a subpopulation of cells in TDL (<0·1%) bearing the injected antigen. These probably correspond to the functionally-active population of AG-L cells in TDL reported previously (Bell, 1979). The cells carrying antigen were characterized as large, non-specific esterase positive, phagocytic cells, many of which adhered to plastic and resisted whole body irradiation, and were thus deemed to be of macrophage lineage. Ag-L cells were identified in blood and lymph of both rats and mice by using several different thymus-dependent antigens and were able to take up more than one antigen simultaneously.

Macrophages are rarely encountered in efferent lymph (Gowans, 1957; Roser, 1970, 1976; Bell & Shand, 1972), a fact which prompted us to consider the physiological significance of the findings. It was conceivable that Ag-L cells gained entrance to lymph around the cannula during the ensuing inflammatory response following surgery. However, this seems unlikely for two reasons. Cells entering the duct from the peritoneal cavity would have to migrate against the pressure gradient of lymph; in fact, an imperfectly secured cannula results in an accumulation of ascitic lymph at the expense of external drainage. Secondly, the acute inflammatory response around the duct which follows the surgical intervention quickly subsides and the area is rapidly sealed over with fibrin, whereas Ag-L cells are detected in lymph 2, 3 and 4 days later. Supporting but not proving the physiological migration of Ag-L cells was the demonstration of radiolabelled antigen-bearing cells in blood. Were it not for the highly immunogenic nature of this subpopulation they might easily have been overlooked; a frequency of 1/1000–1/5000 is easy to dismiss under normal circumstances. The consistent demonstration of Ag-L cells both functionally and microscopically, their presence in both rats and mice, and the recent confirmation by Anderson & Warren (1982) of small numbers of macrophages in cultures of TDL suggest to us that the subpopulation may be physiologically relevant—possibly in the transport of antigen to distant lymphoid tissue.

There is no direct evidence at present on the immediate origin of Ag-L cells in thoracic duct lymph. They could originate from the population of cells free in the peritoneal cavity and migrate via the afferent lymphatic channels draining the posterior abdomen (Olin & Saldeen, 1964). Following this route antigen-carrying cells would have to migrate across the lymph node matrix before emerging in TDL. There is no experimental support for a more direct migration from peritoneal cavity into the cysterni chyli. Alternatively, free antigen could be taken up by macrophages already present in lymph nodes which subsequently migrate to the efferent lymphatics. At present, there is no evidence that would favour one pathway more than the other.

The importance of accessory cells in initiating an immune response was established in the late 1960s (Unanue & Askonas, 1968). The more recent findings of a correlation between T-cell stimulation and Ir gene products on accessory cells (Yamashita & Shevach, 1977) prompted the examination of Ag-L cells for Ia using the monoclonal antibody OX4 which detects a common Ia antigen on all rat strains (McMaster & Williams, 1979). The absence of Ia from the surface of Ag-L cells was somewhat unexpected. However, the expression of Ia on macrophages is variable and can be modified by antigens, lymphokines, activated T cells or variations in culture conditions (Beller, Kiely & Unanue, 1980; Lee & Wong, 1980; Steinman, Nogueira, Witmer, Tydings & Mellman, 1980). Furthermore, in one experimental situation, surface Ia disappeared following acute irradiation (Beller & Unanue, 1981). Thus it is possible that the irradiation given to the donors of Ag-L cells for surface phenotyping could have affected our results. However, this seems unlikely since in many of the Ag-L cell donors 6–8 weeks had elapsed following irradiation, and would have provided ample time for macrophage replacement before thoracic duct cannulation. It was also apparent that irradiation did not impair the antigen presenting function (Table 5). Furthermore, recent experiments with non-irradiated donors have substantiated the Ia negative status of adoptively transferred Ag-L cells, even after they have localized in the spleen (to be published).

Whether the Ag-L cell is related to other antigen-presenting cells (e.g. the dendritic/veiled cell) is unknown but important differences exist. Unlike the essential macrophage-like qualities of the Ag-L cell, the non-phagocytic, temporarily adherent, strongly Ia positive surface of dendritic/veiled cells stands in sharp contrast. The latter cells are clearly associated with the proliferation stage of T-cell stimulation (Steinman & Witmer, 1978; Klinkert, LaBadie, O'Brien, Beyer & Bowers, 1980; Sunshine, Katz & Feldmann, 1980) but unlike the Ag-L cell show little or no propensity to take up antigen (Silberberg-Sinakin

et al., 1976; Kelly, Balfour, Armstrong & Griffiths, 1978). The anatomical counterpart of the dendritic/veiled cell is thought to be the interdigitating cell which occupies the T-dependent areas of lymph nodes (Kelley *et al.*, 1978; Drexhage *et al.*, 1979). However, our recent studies indicate that mobile Ag-L cells migrate preferentially to the splenic red pulp (to be published). Ag-L cells may have more similarities with one of the distinct macrophage populations in the spleen (marginal zone and red pulp macrophages) recently identified by Humphrey & Grennan (1981) on the basis of differential uptake of neutral or acidic polysaccharides.

Present evidence suggests, therefore, that there may be several distinct accessory cells required for lymphocyte activation which could have evolved to meet the diverse needs of a specialized and complex immune system.

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