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Comparative migration of T- and B-lymphocyte subpopulations into skin inflammatory sites

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Summary. Homing of ^{51}Cr-labelled spleen and lymph node-derived T- and B-cell subpopulations into inflammatory sites in the skin, as well as into lymphoid and non-lymphoid organs, was studied in outbred and inbred guinea-pigs. The early progeny of activated T cells were demonstrated in immunologically-mediated inflammation of the skin, for example a cell-mediated immune reaction and a reversed passive Arthus reaction. In contrast, spleen and lymph node-derived B cells were virtually unable to enter inflammatory sites. Organ-specific homing of T and B cells was detected. The significance of these observations for the interpretation of results of lymphoid cell transfers in guinea-pigs is discussed.

INTRODUCTION

T lymphocytes are often present in inflammatory reactions in higher numbers than might be expected from their contribution to the lymphocyte population of the peripheral blood (van Boxel and Paget, 1975; van de Putte, Meyer, Lafeber, Kleinjan & Cats, 1976; von Willebrand, Toetterman, Horsmanheimo, Kuestala & Haeyry, 1978; van den Berg, van Maarsseveen, Mullink & Scheper, 1980). A similar T-cell predominance can also be found in normal body fluids, e.g. the peritoneal fluid (Manconi, 1977) and lymph draining normal skin (Scollay, Hopkins & Hall, 1976).

These observations suggested to us that B lymphocytes do not extravasate as readily as T cells. In agreement with that is the observation that B cells possess a lower intrinsic locomotor capacity than T cells *in vitro* (Scheper, van Maarsseveen and van Dinther-Janssen, 1980). Results of transfer studies to characterize the recirculation of radiolabelled subpopulations of lymphoid cells through lymphoid organs also support this view (Sprent, 1973; Nieuwenhuis & Ford, 1976; Frost, 1978).

In this report we describe the homing properties of purified subpopulations of guinea-pig T and B cells into immunologically-mediated inflammation in the skin, as well as into lymphoid and non-lymphoid organs.

Similar studies have contributed to our understanding of T-cell recirculation in mice (Asherson & Allwood, 1972; Rose, Parrott & Bruce, 1978).

B-cell recirculation, however, is much less well documented. In addition, no information is yet available on the differential migratory capacity of T and B lymphocytes *in vivo* in guinea-pigs. That information is needed as lymphoid cell transfers have been used in experiments with guinea-pigs for many years. Cell transfers are still of paramount importance in studies of the expression and regulation of cell-mediated immunity (Parker, Turk & Scheper, 1976; Askenase, 1976; Polak, 1980).

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MATERIALS AND METHODS

Animals

Outbred female albino guinea-pigs, weighing 250–300 g were used in most experiments. Inbred (strain 13) guinea-pigs were kindly provided by Dr H. A. Brouwer (Dept. Exp. Geneeskunde), strain 2 guinea-pigs were a gift of Dr L. Polak (Hoffmann La Roche, Basel).

Preparation of lymphocyte cell suspensions

Guinea-pigs were sensitized by epicutaneous application of 0·1 ml of a 4% (w/v) solution of 2.4-dinitrochlorobenzene (DNCB) in 96% ethanol on both ears. Lymph nodes, unless otherwise stated, were always obtained 5–7 days after DNCB painting. Lymphoid cell suspensions were prepared from groups of at least four donors by teasing the pooled draining auricular, cervical and tracheal lymph nodes, spleens or thymuses, in RPMI 1640 (Gibco Europe, Glasgow) filtering through nylon gauze and washing twice. Cells, suspended in RPMI 1640 containing 10% heat-inactivated foetal calf serum (FCS) at a concentration of 5×10^7 cells/ml, were used for separation.

Separation of T and B lymphocytes

T- and B-cell subpopulations were prepared as described earlier (Scheper *et al.*, 1980). In brief, one passage through nylon wool columns was used to obtain enriched T-cell suspensions. The adherent fraction containing B cells was removed by repeatedly compressing the nylon wool columns with forceps. The columns were washed three times with RPMI 1640 containing 10% FCS. The cells obtained were washed, pooled and incubated with papain-treated rabbit red blood cells (RRBC), after which rosette forming cells (T lymphocytes) and dead cells could be removed by centrifugation through a Ficoll-Hypaque cushion (density 1·085 g/ml). The interface cells (B lymphocytes) were collected, washed three times and adjusted to a concentration of 10^7 cells/ml in RPMI 1640 containing 15% heat-inactivated guinea pig serum. Cells (3×10^7) were incubated on plastic tissue culture petri dishes (Falcon, 100 × 20 mm) for 90 min at 37° in 5% CO_2 to remove contaminating phagocytic cells. T-cell-enriched cell suspensions contained 65%–75% RRBC-RFC, between 1 and 5% Ig-positive cells by immunofluorescence, and less than 0·8% esterase-positive cells. B-cell-enriched cell suspensions contained less than 3% RRBC-RFC, 65%–80% Ig-positive cells and less than 5% esterase-positive cells. Yields varied for both T and B cells from 20% to 30%; viability was always higher than 85% as determined by eosin exclusion. In some experiments B-cell-enriched suspensions were prepared by rosette formation of surface Ig-positive cells. Rabbit anti-guinea-pig Ig-conjugated SRBC for rosetting were prepared according to Gronowicz, Coutinho & Melchers (1976). Selection of B cells as B-RFC was performed using a Ficoll-Hypaque cushion (density 1·085 g/ml, 5 min, 500 *g*). After NH_4Cl-induced lysis of the red cells B-cell preparations were washed three times with RPMI 1640 medium.

Preparation of T cells from chronic peritoneal inflammatory exudates

Chronic peritoneal inflammatory exudates were induced by repeated intraperitoneal injections of 10 μg PPD (RIV, Bilthoven, Holland) into guinea-pigs sensitized to FCA (containing mycobacterial strain H37Ra, Difco). T cells were purified from the chronic peritoneal exudate cell suspensions by nylon wool filtration as described earlier (van den Berg, van Tuyl, Sminia, Jentjens & Scheper, 1981).

Radioactive labelling of donor lymphocytes

Purified T and B cells were incubated with 50 μCi/ml Na_2 [^{51}Cr]O_4 (Amersham) in RPMI 1640 containing 5% FCS (10^8 cells/ml, 30 min, 37°). After labelling cells were washed three times and adjusted to a concentration of 5×10^7 viable cells/ml. No differences in ^{51}Cr uptake (average ± SD: 14 ± 6%) between the different cell suspensions could be detected. Viability always exceeded 85% as determined by eosin exclusion.

Lymphocyte recipients

Guinea-pigs were sensitized by epicutaneous application of a 4% solution of 2,4-dinitrochlorobenzene (DNCB) in 96% ethanol. Two–three weeks after sensitization cell recipients were skin tested on the flanks with 50 μl 0·3% DNCB (intensity of erythema at 20 hr: 1·2 ± 0·3, $n = 50$). Two–four hours after challenging, 5×10^7 radiolabelled cells were injected i.v. into the ear veins, after which the recipients were killed with ether 5–20 hr later.

In some experiments reversed passive Arthus reactions were elicited by skin testing with a 1:10 dilution of a high-titred anti-ovalbumin serum. Five milligrams ovalbumin was added to the labelled cell inoculum (increase in skin thickness at 6 hr: 1·8 ± 0·3 mm, at 20 hr: 0·9 ± 0·2 mm).

Some groups of recipients received intravenous

injections of 1 ml of a 10% suspension of colloidal carbon (Pelikan C11/1431a, Gunther Wagner) in 0·9% sodium chloride at a concentration of 50 mg/ml, 15–30 min before cell transfer (Morgan & Holt, 1979). Other groups received intraperitoneal injections of 300 mg/kg cyclophosphamide (Endoxan, Asta, Brackwede, Germany) 3 days before cell transfer (Poulter & Turk, 1972).

Sample preparation

Samples of blood, lungs, liver, spleen, axillary lymph nodes, bone marrow and urine were collected. In addition, 25 mm diameter skin pieces were taken including both normal untreated skin and inflammatory sites. Data on skin test localization, unless otherwise stated, refer to DNCB skin tests. Radioactivity was measured in a gamma scintillation counter (LKB). The amount of radioactivity in each organ is expressed as a percentage of the injected dose. Data on blood localization refer to cell-bound radioactivity. Plasma-bound radioactivity in T-cell transfer experiments never exceeded 12% of the total blood values (0·5% of the injected dose). For non-T cells and heat-killed T cells these percentages were markedly higher: 50% (1·9%).

RESULTS

Distribution of syngeneic, allogeneic and xenogeneic lymph node-derived T lymphocytes

Dependence on the strain combinations of the distribution of lymph node-derived T cells into recipients was studied. Figure 1 shows the organ distribution 20 hr after i.v. injection of syngeneic (strain 13 into strain 13) and allogeneic cells (strain 2 into strain 13, and outbred). In all three combinations an equally strong skin test localization was observed. With the exception of the 2–13 combination, both the outbred and syngeneic combinations showed similar lymph node localization. Allogeneic cells generally accumulated more in the liver.

Most data in the following experiments were obtained from our colony of outbred guinea-pigs. In

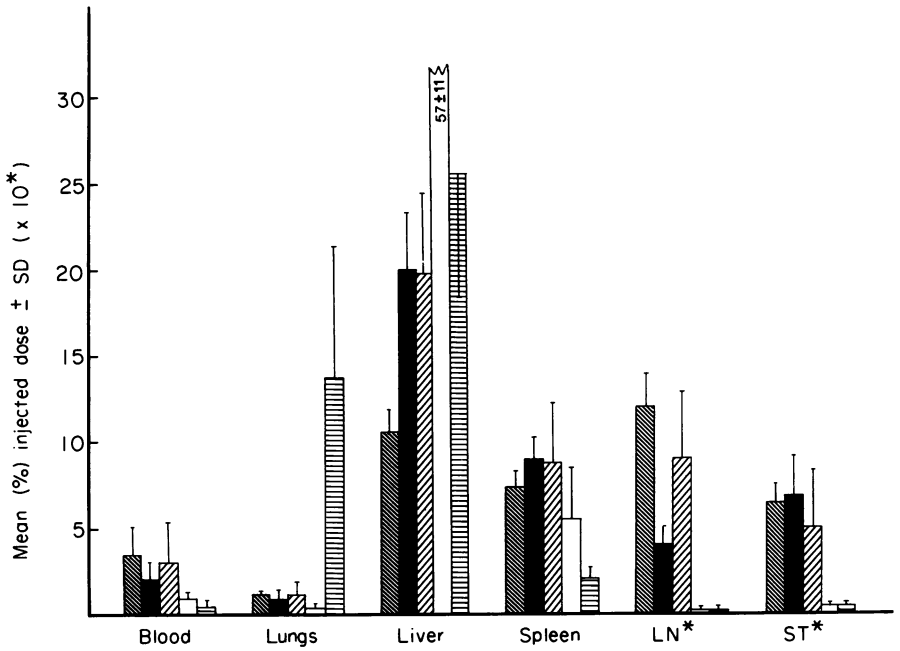

Figure 1. Recovery of radioactivity in guinea-pigs 20 hr after the intravenous injection of 5×10^7 ^{51}Cr-labelled syngeneic (13–13, ▧) or allogeneic (2–13, outbred, ■) lymph-node-derived T lymphocytes (▨), xenogeneic human tonsil-derived T lymphocytes (□) or heat-killed (outbred) lymphocytes (▤). LN, accessory axillary lymph node; ST, DNCB skin test site (see Materials and Methods). Control skin samples never contained more than 0·05% of the injected dose. Each value represents the mean ± SD of at least six guinea-pigs.

outbred guinea-pigs, T-cell distribution at 5 hr (data not shown in the figure) was high in the spleen ($17 \pm 5\%$, $n = 5$) and low in lymph nodes and DNCB skin tests ($0{\cdot}10 \pm 0{\cdot}03\%$ and $0{\cdot}05 \pm 0{\cdot}02\%$, respectively, $n = 5$) when compared with data obtained at 20 hr.

The marked capacity of the liver to retain non-histocompatible lymphoid cells was particularly evident from cell transfers using T cells derived from human tonsils. These xenogeneic lymphocytes were unable to migrate into lymph nodes or into a skin inflammatory site. Heat-killed, allogeneic T lymphocytes also failed to show functional migration.

Distribution of T cells derived from different organs or a chronic inflammatory reaction

T cells derived from lymph nodes and spleen were equally able to migrate into an inflammatory site in the skin (Fig. 2). In contrast, both cell populations exhibited clear organ-specific homing.

Thymocytes were incapable of entering either lymph nodes or the skin reaction site. The major part of the thymocyte inoculum apparently localized in the liver and spleen. Cells derived from lymph nodes, spleen or thymus did not show significant homing into the thymus (values never exceeded $0{\cdot}06\%$ of the injected dose, data not shown in the figure).

T cells that had been purified from chronic peritoneal exudates showed a strong propensity to migrate into inflammatory sites in the skin. On average 7% of the inoculum could be recovered from a DNCB contact reaction. A dichotomy between skin test and lymph node migration pathways was evident from the lack of increase in lymph node localization of these chronic exudate-derived T cells as compared with lymph node or spleen-derived T lymphocytes.

Distribution of lymph-node-derived T cells at different stages of activation

In most transfer studies of CMI in guinea-pigs, T cells are obtained 1–4 weeks after sensitization. Because T cells obtained from lymph nodes 4 days after contact sensitization of mice show optimum capacity to home to inflammatory sites (Asherson & Allwood, 1972), we decided to study the time course of T-cell migration

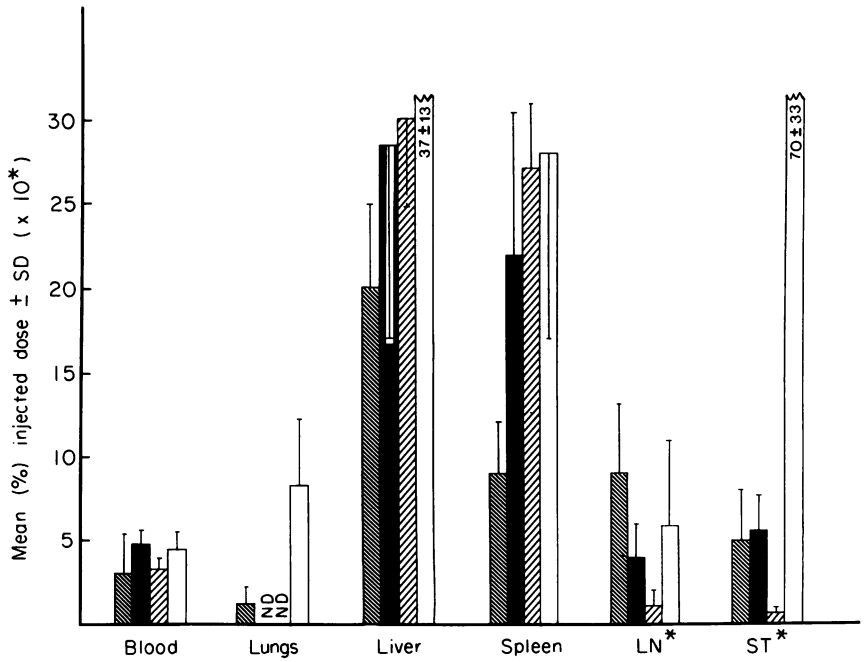

Figure 2. Recovery of radioactivity in guinea-pigs 20 hr after the intravenous injection of 5×10^7 ^{51}Cr-labelled lymph node (▨), spleen (■), thymus (■) or chronic peritoneal exudate-derived T lymphocytes (□). LN, accessory axillary lymph node; ST, DNCB skin test site (see Materials and Methods). Each value represents the mean ± SD of at least six guinea-pigs.

into a skin inflammation in guinea-pigs. Groups of guinea-pigs were painted with a sensitizing dose of DNCB, after which lymph nodes were removed at 0, 2, 4, 6, 8 and 12 days. Purified T cells were counted for blast cell percentages (by light microscopy using a May-Grunwald-Giemsa stain), radiolabelled with ^{51}Cr and injected intravenously into recipients that had been sensitized to DNCB 10 days earlier.

From the results presented in Fig. 3 it is clear that cell suspensions with relatively low numbers of blast cells (8 days after contact sensitization) showed optimum migration into the inflammatory site in the skin. Essentially similar results were found when the recipients were both sensitized and skin tested with the unrelated contact agent oxazolone (data not shown).

Distribution of B lymphocytes as compared with T lymphocytes

B lymphocytes derived from either lymph nodes or the spleen showed only minimum localization in the skin following i.v. transfer (Fig. 4). Failure to migrate into contact reaction sites coincided with low blood cell counts and a marked accumulation in the liver. Just as T cells, B lymphocytes generally showed clear preferential homing to the organ of origin. Radioactivity in femur (0·5%–2%) and 20 hr urine (4%–8%) was not different in recipients of T or B cells derived from either lymph nodes or the spleen.

The poor migration of B cells into an inflammatory site was not influenced by (see Table 1) various factors like the use of different strain combinations, unstimulated or stimulated lymph nodes, the method of B-cell purification (nylon wool positive, E rosette negative and surface Ig^+, respectively), the cell dose injected (3×10^7–10^8) or the immunological nature of the inflammation: contact sensitivity reaction of the delayed type ($0{\cdot}07 \pm 0{\cdot}03\%$) and reversed passive Arthus reaction ($0{\cdot}05 \pm 0{\cdot}02\%$). For T cells these percentages were $0{\cdot}5 \pm 0{\cdot}2\%$ and $0{\cdot}4 \pm 0{\cdot}2\%$; data not shown in the Table.

An attempt was made to reduce trapping of B cells in the liver which might permit increased blood cell levels and increased localization into the skin. Following colloidal carbon treatment (experiment 5 in Table 1) B-cell localization in the liver was reduced to the level normally seen with T cells ($17 \pm 5\%$). However, blood cell radioactivity remained low, and localization

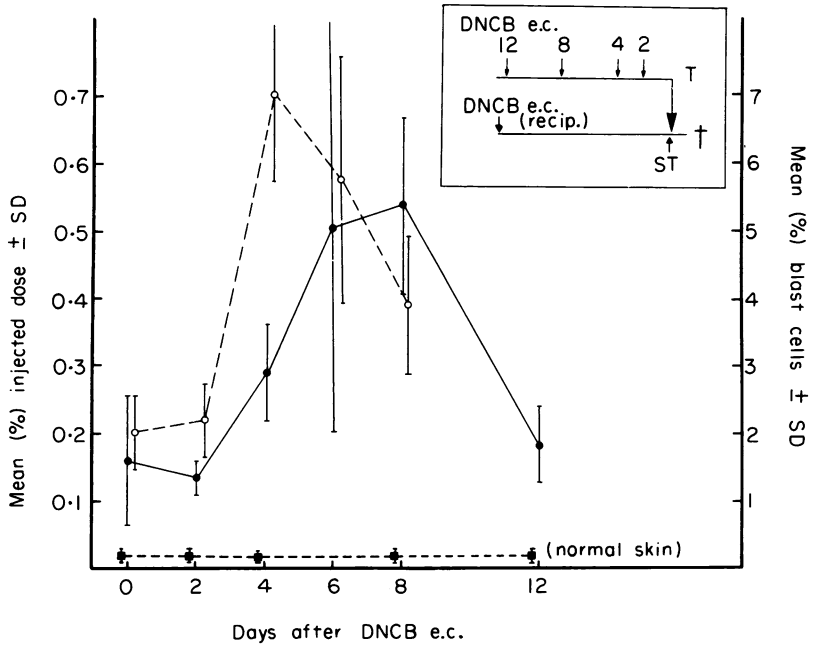

Figure 3. Recovery of radioactivity in a DNCB skin test site 20 hr after the intravenous injection of 5×10^7 ^{51}Cr-labelled T cells derived from lymph nodes 0, 2, 4, 6, 8 and 12 days after epicutaneous sensitization with DNCB (●——●). Percentages of lymphoblasts in these cell suspensions (○- - -○). Each value represents the mean ± SD of at least six guinea-pigs.

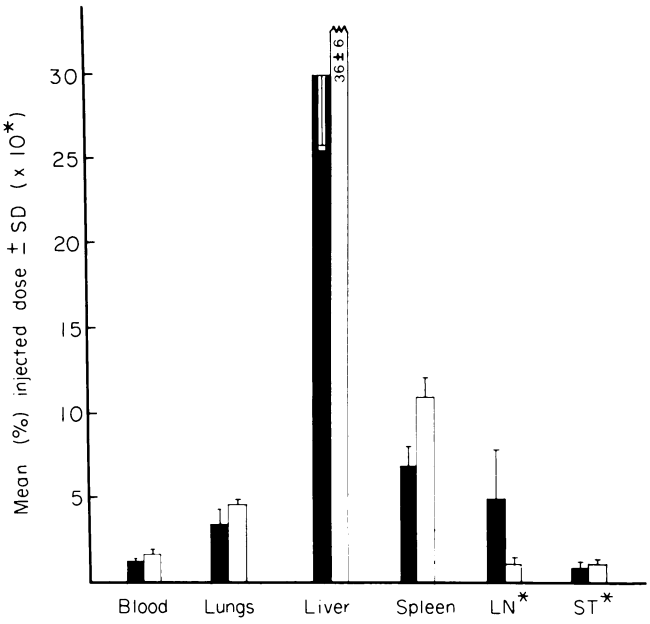

Figure 4. Recovery of radioactivity in guinea-pigs 20 hr after the intravenous injection of 5×10^7 ^{51}Cr-labelled lymph node- or spleen-derived B lymphocytes. LN, accessory lymph node; ST, DNCB skin test site (see Materials and Methods). Control skin samples never contained more than 0·05% of the injected dose. Each value represents the mean ± SD of at least six guinea-pigs. BLN (■); B spleen (□).

Table 1. Distribution of ^{51}Cr-labelled lymph-node-derived B cells in recipient guinea-pigs

Experiment*		Blood	Liver	Lymph node	Skin test site‡
1. Strain combination	Syngeneic (strain 13)	0·6 ± 0·1†	16 ± 5	0·4 ± 0·2	0·07 ± 0·03
	Allogeneic (outbred)	1·1 ± 0·3	30 ± 4	0·5 ± 0·3	0·07 ± 0·03
2. Donor lymph nodes	Normal, unstimulated	0·8 ± 0·2	25 ± 7	0·5 ± 0·2	0·06 ± 0·04
	6 days after DNCB e.c.	1·1 ± 0·3	30 ± 4	0·5 ± 0·3	0·07 ± 0·03
3. B-cell purification	Nylon wool$^+$, E$^-$	1·1 ± 0·3	30 ± 4	0·5 ± 0·3	0·07 ± 0·03
	Surface Ig$^+$	0·6 ± 0·1	23 ± 4	0·6 ± 0·2	0·09 ± 0·01
4. B-cell dose	3×10^7 per recipient	0·7 ± 0·1	18 ± 8	0·4 ± 0·2	0·09 ± 0·06
	10×10^7 per recipient	0·5 ± 0·1	19 ± 9	0·5 ± 0·2	0·10 ± 0·09
5. Phagocyte blocked recipients	Control	1·1 ± 0·2	30 ± 4	0·8 ± 0·2	0·13 ± 0·08
	Carbon-pretreated	1·2 ± 0·5	17 ± 3	0·7 ± 0·1	0·12 ± 0·04
6. Lymphopenic recipients	Control	0·9 ± 0·2	26 ± 4	0·5 ± 0·3	0·08 ± 0·04
	Cy-pretreated	0·5 ± 0·1	27 ± 3	0·3 ± 0·2	0·09 ± 0·03

* Unless stated otherwise, B cells were purified as nylon wool adherent, E-rosette negative cells from lymph nodes obtained from outbred guinea-pigs 6 days after painting with DNCB. Generally each recipient received 5×10^7 B cells intravenously.

† Each value represents the arithmetic mean ± SD for at least six guinea-pigs.

‡ Type IV contact reaction to DNCB, see Materials and Methods.

in the skin was not increased by this procedure. With recipients, pretreated with cyclophosphamide, it was shown that the poor homing of B cells to skin test reactions was not due to competition with the recipients own lymphoid cells (experiment 6 in Table 1). Injection of ^{51}Cr-labelled lymph node-derived B cells into lymphopenic guinea-pigs did not increase migration into the skin test. On the other hand, T cells were increased in the blood (from $3{\cdot}5 \pm 0{\cdot}8\%$ in control recipients till $5{\cdot}6 \pm 0{\cdot}4\%$ in recipients pretreated with CY). An increased migration of T cells to skin test sites was also observed ($0{\cdot}5 \pm 0{\cdot}2\%$ and $1{\cdot}6 \pm 0{\cdot}6\%$, respectively, data not shown in the table).

DISCUSSION

Several studies in the past have made it clear that successful transfer of cell-mediated immunity (CMI) in guinea-pigs can be accomplished with outbred guinea-pigs (Landsteiner & Chase, 1942; Katz, 1974). Some degree of genetic relationship however was found to be indispensable (Turk & Leibowitz, 1964). That dependence has now recently been attributed to Ia-restricted antigen presentation (Shevach, 1978). In agreement with these data we found equal migration of T cells derived from lymph nodes into contact sensitivity reactions in the skin in allogeneic and syngeneic combinations. In contrast, strain 2 T-cell recirculation through strain 13 lymph nodes was reduced, suggesting Ia restriction as has been described in both rats (Gowans, 1959) and mice (Zatz, Gingrich & Lance, 1972; Gillette, 1979). Xenogeneic T cells did not recirculate through lymph nodes nor migrate into skin test sites.

Large, S-phase T blasts, present in peripheral lymph nodes 3–4 days after contact sensitization, have been identified as the lymphoid cells with optimum capacity to enter inflammatory sites (Asherson, Allwood & Mayhew, 1973; Allwood, 1975; Rose *et al.*, 1978). Small thoracic duct-derived lymphocytes have also been shown to migrate into CMI lesions (Rannie, Smith & Ford, 1977). Results presented here from experiments with guinea-pigs favour the hypothesis that immediate descendants of T-cell blasts, presumably small lymphocytes, are the cells that migrate into inflammatory sites. Such T lymphocytes are known to accumulate in the peritoneal cavity of recently sensitized animals by induction or either immunologically or non-immunologically induced peritoneal inflammatory reactions (Lefford, 1980). Upon intravenous infusion into recipients, peritoneal exudate lymphocytes are extremely potent mediators of delayed hypersensitivity (Polak, 1980). Lymphocytes isolated from chronic peritoneal inflammatory reactions also have a marked capacity to migrate to sites of inflammation (Fig. 2).

Our data do not support a relationship between small lymphocyte migration through post-capillary high endothelial venules within the lymph nodes and through small venule endothelia present in CMI lesions (Rannie *et al.*, 1977): neither chronic peritoneal exudate lymphocytes, nor lymph node lymphocytes prepared 8 days following stimulation, showed an increased tendency to localize into peripheral lymph nodes. It has been suggested that separate factors exist that select cells for localization into CMI lesions and peripheral lymph nodes (Issekutz, Chin & Hay, 1980). This is also suggested from the present study by the similar capacity of spleen and lymph node-derived T cells to enter CMI lesions, whereas the latter T cells show markedly stronger lymph node localization. The selective migration of lymphoid cells to their organ of origin has been attributed to organ-specific endothelial cell determinants for lymphocyte binding (Butcher, Scollay & Weissman, 1980). If similar migration-regulating endothelial cell determinants are present in CMI lesions, our data would require at least three different receptors to explain the various patterns of T lymphocyte homing.

Whereas it has been generally accepted that B cells recirculate more slowly than T cells, the differential capacity of B and T cells to migrate into inflammatory sites is less well documented. From the present study in guinea-pigs, it appears that both splenic B cells and lymph node-derived B cells are virtually unable to migrate into skin reactions. Lymph node-derived B-cell homing was similarly poor into contact sensitivity reactions as into a reversed passive Arthus reaction. This observation may have important implications for the interpretation of data obtained from transfers of lymphoid cells. Failure to transfer contact sensitivity in guinea-pigs with purified B cells derived from lymph nodes (Scheper, unpublished results) could be explained by the inability of those B cells to enter skin test sites.

In studies to assess the role of different lymphocyte subpopulations in cell-mediated immune reactions care should be taken to minimize the effects of differences in migration behaviour. This could be accomplished by using local passive transfers (Stash-

enko, Bhan, Schlossman & McCluskey, 1977) or *in vitro* assays (Scheper & Polak, 1981).

In conclusion, the results of this study demonstrate the superior migration efficiency of T-cell subpopulations, as compared with B cells. In acute inflammatory reactions this dichotomy may account for the large predominance of T lymphocytes.

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