THE DISTRIBUTION OF ^{51}CR-LABELED LYMPHOCYTES INTO ANTIGEN-STIMULATED MICE

Lymphocyte Trapping

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The cellular events related to the induction of humoral and cell-mediated immunity involve antigen recognition, blast transformation, and multiplication of lymphoid cells in lymph nodes and spleen. The early histological changes in lymph nodes draining the site of a skin graft (1, 2), particulate antigen injection (3, 4), adjuvant stimulation (5), or application of skin-sensitizing chemicals (6–9) have been shown to involve mainly proliferation of thymus-derived lymphocytes in the paracortical regions adjacent to postcapillary venules. Before this mitotic activity, 1–3 days after antigenic challenge, a "trapping" of circulating lymphocytes has been reported to occur (10–14); this phenomenon has also been demonstrated in spleen (15, 16).

Since recruitment of lymphocytes may be amongst the earliest of cellular events in the initiation of immune responses, it was of interest to investigate this phenomenon quantitatively. The kinetics of trapping were studied by following the distribution of ^{51}Cr-labeled lymphocytes in groups of syngeneic mice which had been previously challenged with sheep erythrocytes, *Salmonella typhi* H antigen, keyhole limpet hemocyanin, allogeneic, or xenogeneic skin. Previous work has demonstrated the feasibility of using the system of ^{51}Cr-labeled lymphocyte migration to quantitate changes in lymphoid populations (17–19).

Methods

Animals.—Pathogen-free mice were obtained from the Jackson Laboratories (Bar Harbor, Maine) and Lewis rats from Microbiological Associates (Bethesda, Md.); CBA/J mice were routinely used as lymphocyte donors and recipients. For experiments involving grafting, C57BL/6J mice or Lewis rats were used as tail skin donors, and CBA/J mice as skin recipients.

Cell Suspensions.—Single cell suspensions were prepared as described previously (18) from the pooled axillary, brachial, inguinal, and mesenteric nodes, by gently pressing the organs

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through a nylon sieve into medium 199 containing 5% fetal calf serum[1] (FCS)[2] (Microbiological Associates). Cell clumps were removed by filtration through washed cotton wool, and the resulting suspensions were counted, checked for cell viability by trypan blue dye exclusion, centrifuged, and resuspended in medium 199 with 15% FCS. Cells were labeled according to the method of Bainbridge and Gowland (20) at a concentration of 25 μCi/10^8 cells per ml, with radiochromium, supplied as sodium chromate-^{51}Cr in sterile isotonic solution (Amersham-Searle Corp., Chicago, Ill.). After 30 min of incubation at 37°C the labeled cells were washed once and resuspended in medium 199 with 15% FCS for injection into mice.

Immunization.—Groups of three mice were injected either intravenously, intraperitoneally, or subcutaneously on the anterior and posterior left flank with sheep erythrocytes (SE) (Microbiological Associates), agglutinable *Salmonella typhi* H antigen (H) (Burroughs-Wellcome & Co., Ltd., London, England) or keyhole limpet hemocyanin (KLH) (Mann Research Labs, Inc., New York). To study primary responses, doses of 5×10^8 SE, 0.2 ml H, or 100 μg KLH were used. For secondary responses, mice were given a sensitizing injection intraperitoneally of 5×10^7 SE, 0.2 ml H, or 10 μg KLH, followed 1–2 months later by equal doses of antigen given intravenously, intraperitoneally or subcutaneously.

Skin Grafting.—To study the first set allograft or xenograft response, CBA/J mice were grafted on the left thorax with C57BL/6J or rat tail skin according to the method of Billingham and Medawar (21). For the second set response, CBA/J mice were grafted twice, 3 wk apart, with C57BL/6J skin on the left thorax.

Experimental Design.—

Response to antigens: 10×10^6 ^{51}Cr-labeled lymphocytes were injected intravenously into groups of three recipients which had received antigen 1, 6, or 24 hr earlier, and into nonstimulated controls. 20–24 hr later, recipients were sacrificed by cervical dislocation and the distribution of lymphocytes was quantitated by assaying the radioactive content of the brachial, axillary, inguinal, and mesenteric lymph nodes, spleen and liver, in a Packard autogamma well spectrometer (Packard Instrument Co., Downer's Grove, Ill.). In groups of animals receiving antigen subcutaneously, the right, left, and mesenteric nodes were sampled separately. Results were first calculated as the per cent of localization of labeled cells in recipient organs relative to the injected dose. The average per cent of recovery for each experimental group was then expressed as a percentage of the recovery in the untreated (control) group.

Response to grafting: Groups of three mice were grafted with either C57BL/6J, rat, or CBA/J (controls) tail skin on the left thorax. At 1–21 days after grafting, labeled lymphocytes were injected intravenously into groups of three mice, and the per cent of distribution of cells was determined after 24 hr in the right and left peripheral nodes, as described above. Results were expressed as the ratio of localization in the left to right nodes.

RESULTS

Effect of Time and Route of Antigen Administration on the Distribution of ^{51}Cr-Labeled Lymphocytes

Primary Response.—When the distribution of lymphocytes was compared in mice receiving SE, H, or KLH with that occurring in controls, significant differences in lymphocyte localization were noted, depending on the route and time of

[1] Previous experiments (unpublished observations) have shown that the addition of FCS or autologous mouse serum to the medium results in comparable distribution patterns of ^{51}Cr-labeled lymphocytes.

[2] *Abbreviations used in this paper:* FCS, fetal calf serum; H, *Salmonella typhi* H antigen; KLH, keyhole limpet hemocyanin; SE, sheep erythrocytes.

antigen administration (Fig. 1). The average per cent of localization of cells in a given organ was compared in groups containing three to nine experimental or control mice. The final results were expressed as a percentage of control values. Levels of significance were determned by use of Student's t test.

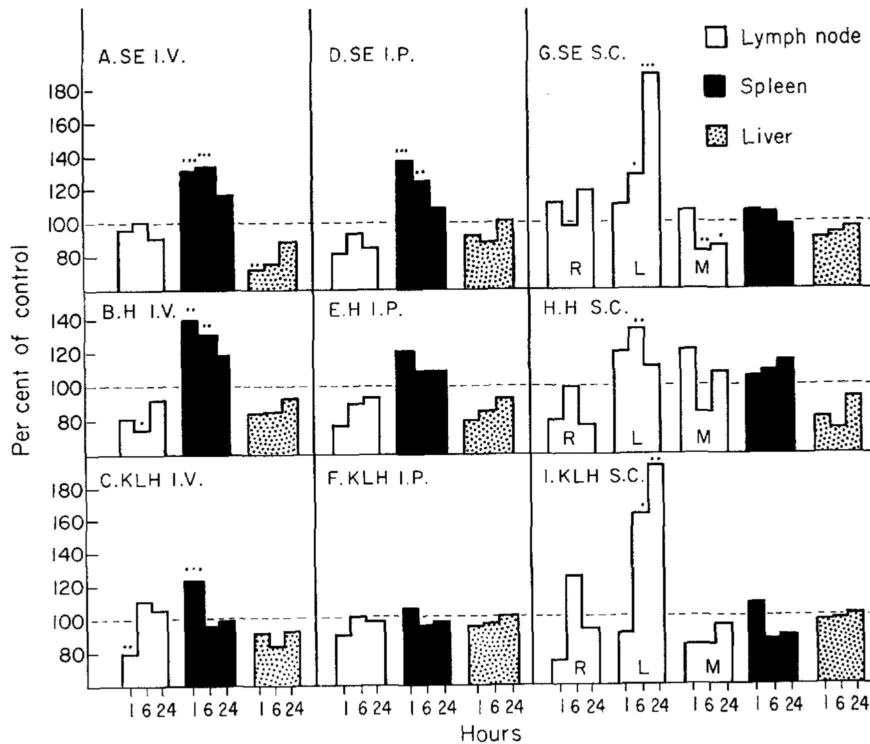

FIG. 1. Effect of time and route of antigen administration on lymphocyte distribution; primary response: ^{51}Cr-labeled lymph node cells were transferred i.v. into recipients 1, 6, or 24 hr after injection of SE, H, or KLH via the i.v., i.p., or s.c. routes. After 24 hr the mean per cent of organ localization of labeled cells was determined in experimental and control (no antigen) groups. The results were expressed as per cent of control organ localization. Significant deviations from controls are indicated by ($P < 0.001$ = • • •, $P < 0.01$ = • •, $P < 0.05$ = •).

Intravenous route (Fig. 1 a, b, c): Significant increments were observed after 24 hr in the splenic localization of labeled cells in recipients previously injected with SE, H, or KLH intravenously. This increased splenic localization was seen when lymphocytes were injected 1 or 6 hr after injection of SE or H and averaged 130% of the control. This effect was less marked in recipients of KLH, and was gone by 6 hr. Concomitant with increased migration of cells to spleen, significantly decreased lymph node localization was observed in intravenous recipients of H or KLH. When labeled cells were injected 1 or 6 hr after intra-

venous injection of particulate antigen (Fig. 1 *a*, *b*), decreased localization in liver of up to 50% of controls also occurred. In all three intravenous groups no significant increment in splenic localization above the control was seen after 24 hr had elapsed between antigen and cell administration.

Intraperitoneal route (Fig. 1 d, e, f): After intraperitoneal injection of SE, increased splenic localization similar to that after intravenous antigen injection was again observed, concomitant with significantly depressed distribution of cells to lymph node and liver. The intraperitoneal route of H and KLH injection was less efficient in eliciting trapping of lymphocytes.

Subcutaneous route (Fig. 1 g, h, i): The effect of subcutaneous injection of antigen on migration patterns of labeled lymphocytes was different from that of

TABLE I

Effect on Cell Distribution when Antigen is Given after Labeled Lymphocytes

Time of SE* injection	‡ Mean per cent localization in					
	Lymph node		Spleen		Liver	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Controls	13.6	(±0.8)	24.8	(±1.3)	14.3	(±0.9)
0	13.4	(±2.7)	26.5	(±2.4)	9.5	(±1.8)
+1 hr	13.1	(±3.2)	25.0	(±1.6)	9.4	(±0.2)
+4 hr	13.1	(±1.1)	25.5	(±2.9)	11.7	(±1.7)

* 5×10^8 sheep erythrocytes were given i.v. either simultaneously, 1, or 4 hr after cell injection. Controls received no antigen.

‡ Cell localization determined in groups of three recipients 20–24 hr after intravenous injection of labeled cells.

intravenous or intraperitoneal antigen administration. In the intravenous and intraperitoneal routes antigen is presumed to localize mainly in spleen (22, 23), whereas the subcutaneous route should result in antigen localization in the nodes draining the site of injection. As expected, increased lymphocyte migration was found to the left nodes, those nodes draining the site of subcutaneous antigen injection. Localization of labeled cells in the peripheral nodes of recipients of SE or KLH (Fig. 1 *g*, *i*) was approximately twice that of controls when cells were injected 24 hr after subcutaneous antigen administration. Distribution of cells to spleen, liver, and right and mesenteric nodes was not significantly different from controls.

Administration of antigen after injection of labeled cells: When cells were administered either simultaneously or before injection of antigen, and recipients were sacrificed 24 hr later, diminished localization of cells in liver occurred, but no trapping of lymphocytes in spleen was observed (Table I). These results make it likely that some processing of antigen before cell injection must occur in order to demonstrate increased accumulations of lymphocytes in antigen-stimulated organs.

Secondary Response (Fig. 2).—The time and route of antigen administration on lymphocyte trapping was found to be similar in presensitized and nonsensitized mice. Maximal trapping of lymphocytes in spleen occurred when antigen was administered intravenously 1–6 hr before cell infusion (Fig. 2 *a*, *b*, *c*). Maximal trapping of lymphocytes in draining lymph nodes occurred when antigen was

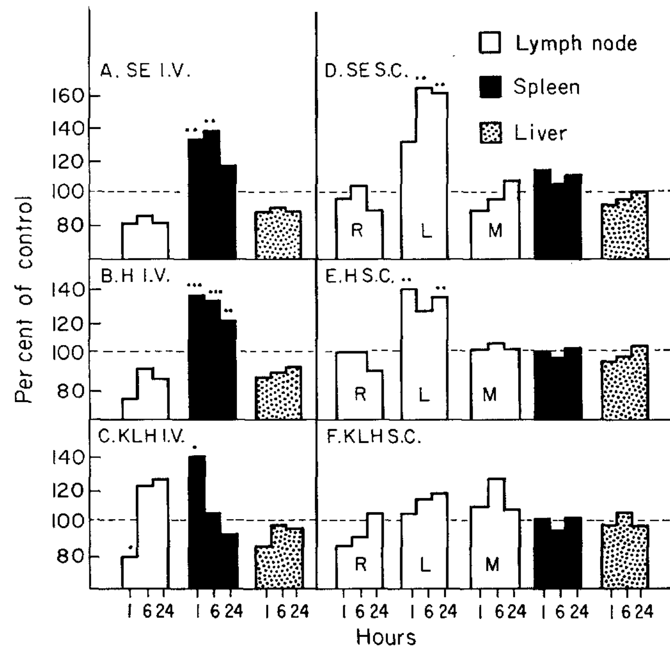

FIG. 2. Effect of time and route of antigen administration on lymphocyte distribution; secondary response: ^{51}Cr-labeled lymph node cells were transferred i.v. into presensitized recipients 1, 6, or 24 hr after challenge with SE, H, or KLH via the i.v. or s.c. routes. After 24 hr the mean per cent of organ localization was determined in experimental and control (no antigen) groups. The results were expressed as per cent of control organ localization. Significant deviations from controls are indicated by ($P < 0.001$ = • • •, $P < 0.01$ = • •, and $P < 0.05$ = •).

administered subcutaneously (Fig. 2 *d*, *e*, *f*). The major difference between presensitized and virgin mice was found in the antigen dose required to elicit the same magnitude of trapping; in the secondary response groups, 10-fold less SE and KLH were used.

Effect of Antigen Dose on Migration of Lymphocytes into Normal and Presensitized Antigen-Stimulated Recipients

The migration patterns of ^{51}Cr-labeled lymph node cells from normal donors were determined 24 hr after injection in untreated and presensitized recipients

of antigen, and in control groups. Antigen was administered intravenously 1 hr before injection of cells, since these conditions were consistently shown to cause increased splenic localization at the antigen doses previously studied. The doses used were, SE: 5×10^9, 5×10^8, 5×10^7, and 5×10^6; H: 0.2 ml of 10x (concentrated by centrifugation at 2500 rpm for 30 min), 1x, and 0.1x standard ag-

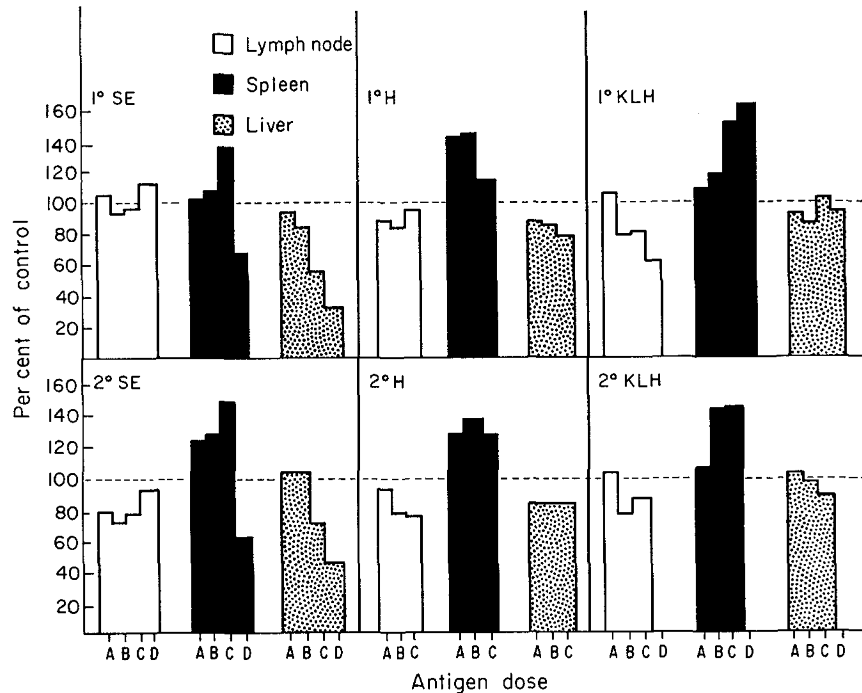

FIG. 3. Effect of antigen dose on lymphocyte distribution in normal and presensitized recipients. ^{51}Cr-labeled lymph node cells were transferred i.v. into recipients 1 hr after i.v. injection of various doses of SE, H, or KLH. After 24 hr the mean per cent of organ localization was determined in experimental and control (no antigen) groups. The results were expressed as per cent of control organ localization.

Dose $A = 5 \times 10^6$ SE, 0.1 x H, or 1 μg KLH; $B = 5 \times 10^7$ SE, 1.0 x H, or 10 μg KLH; $C = 5 \times 10^8$ SE, 10.0 x H, or 100 μg KLH; $D = 5 \times 10^9$ SE, or 1000 μg KLH.

glutinable suspensions of *S. typhi*; and KLH: 1000, 100, 10, and 1 μg. The results are shown in Fig. 3 expressed as percentages of control groups.

In previously unsensitized mice, elevated spleen localization was seen in recipients of 5×10^8 SE, 0.1x and 1.0x H, and 100 μg KLH. Similar trapping of lymphocytes could be elicited with 10-fold lower doses of SE or KLH in presensitized recipients. The lowest doses of SE (5×10^6) and of KLH (1 μg) caused no significant deviation from the lymphocyte migration patterns observed in nonstimulated control recipients, and thus served as a check for any nonspecific

effects. The highest dosages of particulate antigen (5×10^9 SE, 10x H) had an opposite effect from the intermediate dosages on the distribution of lymphocytes administered 1 hr later. Injection of 5×10^9 SE into nonsensitized mice resulted, for example, in elevated lymph node localization and markedly depressed spleen localization (66% of control). The 10-fold lower dose (5×10^8 SE) produced diminished lymph node localization and elevated spleen localization (136% of control). The highest doses of particulate antigen consistently produced diminished liver localization of as much as 30% of controls.

Thus, up to a critical dose, increasing antigen will produce increased spleen localization and decreased lymph node localization. Large doses of particulate antigen will cause the reverse pattern, i.e., increased distribution of labeled cells to lymph node and decreased distribution to spleen.

To summarize, labeled lymphocytes are trapped in the spleens of normal and presensitized mice when SE, H, or KLH are administered intravenously 1–6 hr before cell infusion, and recipients are sacrificed 20–24 hr later; lymphocytes are trapped in draining lymph nodes of normal and presensitized mice when antigen is injected subcutaneously 6–24 hr before cell infusion, and recipients are sacrificed 20–24 hr later. The only significant difference between presensitized and normal recipients is in the dose of antigen required to demonstrate trapping; 10-fold lower doses are sufficient in presensitized mice.

Time of Recipient Sacrifice.—Since the time interval between injection of antigen and labeled cells proved to be critical in discerning changes in lymphocyte migration patterns, it was necessary to determine whether the 20–24 hr interval between injection of cells and sacrifice of recipients was optimal for detecting changes in migration patterns. Two effects of antigen on lymphocyte migration patterns in antigen-stimulated recipients were studied as a function of time of recipient sacrifice: (*a*) increased splenic localization in presensitized mice by intravenous injection of 5×10^7 SE 1 hr before injection of cells; and (*b*) increased localization in draining nodes of normal recipients by subcutaneous injection of 5×10^8 SE 24 hr before injection of cells. Groups of three recipients were sacrificed 1–72 hr after infusion of labeled cells and mean per cent of localization was determined in lymph nodes, spleen, and liver, and compared with nonstimulated controls.

(*a*) *Increased splenic localization* (*Fig. 4*): The difference in distribution of lymphocytes into spleen and lymph node of antigen-stimulated and control recipients was maximal at 24 hr after cell injection. The sustained retention of cells in spleen of antigen-stimulated recipients parallels a plateau in lymph node localization from 12 to 24 hr, indicating that a dynamic equilibrium between these two compartments was disturbed by intravenous injection of 5×10^7 SE 1 hr before cell injection. By 48 hr, the curves for lymph node and spleen distribution converge in normal and antigen-stimulated recipients. Thus, the trapping of cells in the latter group is transient. The curves for liver localization

in the two recipient groups were parallel and not affected by injection of this dose of antigen.

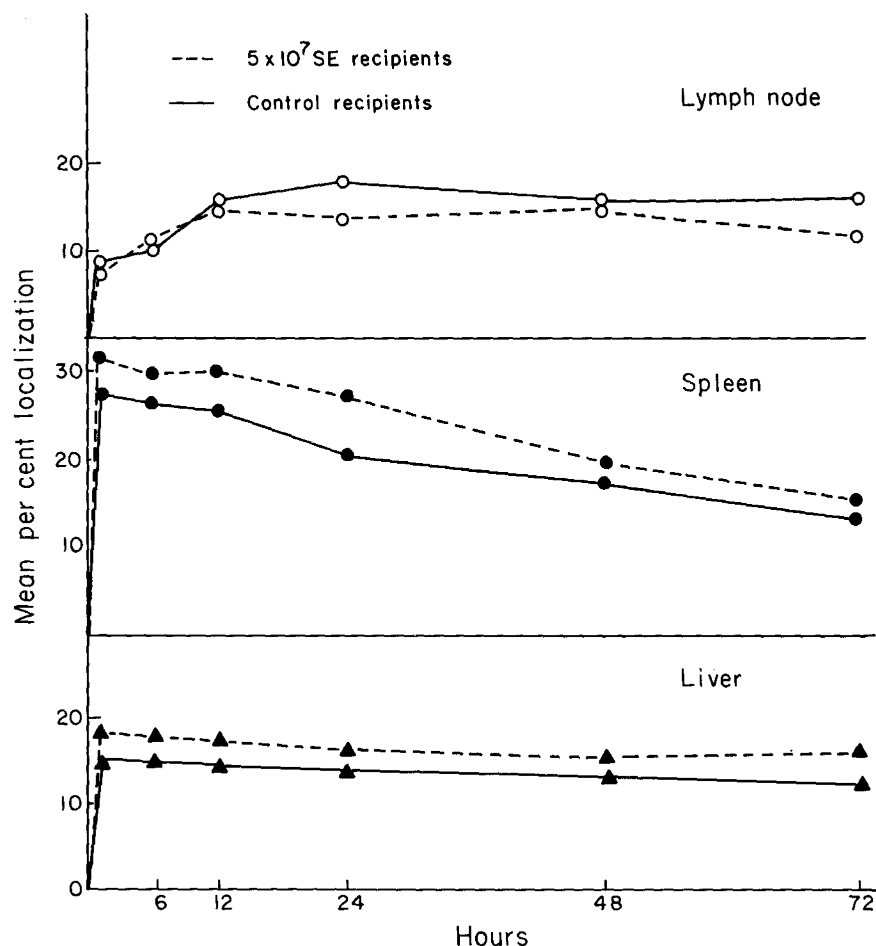

FIG. 4. Kinetics of splenic trapping after i.v. injections of antigen. Labeled lymph node cells were transferred into groups of three presensitized recipients 1 hr after i.v. injection of 5×10^7 SE, or into control recipients. Antigen-stimulated (-----) and control (——) groups were sacrificed 1–72 hr after cell injection, and the mean per cent of localization of cells was determined in lymph node, spleen, and liver.

(*b*) *Increased lymph node localization* (*Fig. 5*): In the left peripheral lymph nodes draining the site where 5×10^8 SE were injected subcutaneously, localization was maximal at 24–48 hr after injection of cells into antigen-stimulated recipients. This effect was compensated for by decreased distribution of labeled

cells to the right peripheral and mesenteric nodes. This elevated nodal localization was also of short duration, the values in normal and antigen-stimulated groups converging 72 hr after cell injection. The dynamics of cell retention in draining lymph nodes after subcutaneous injection of antigen differs from that

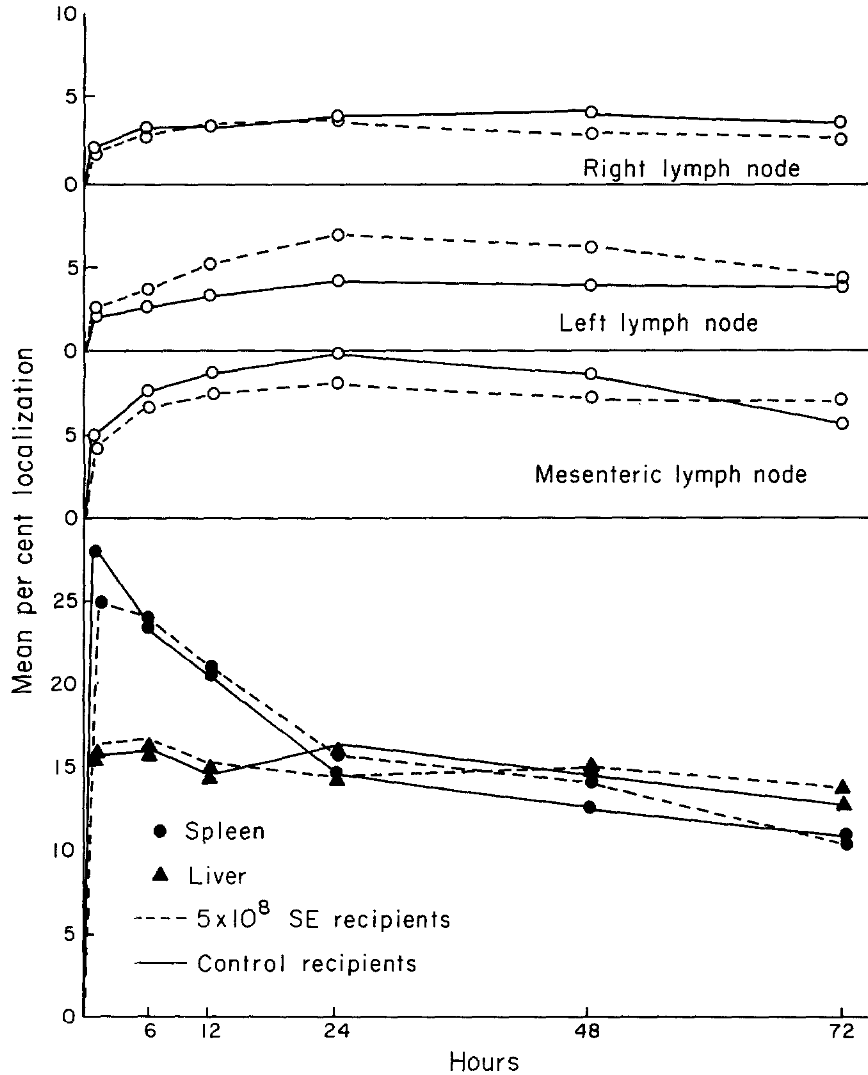

FIG. 5. Kinetics of lymph node trapping after s.c. injection of antigen. Labeled lymph node cells were transferred into groups of three recipients 24 hr after s.c. injection of 5×10^8 SE, on the left flank, or into normal controls. Antigen-stimulated (------) and control (——) groups were sacrificed 1–72 hr after cell injection, and the mean per cent of localization was determined in right, left, and mesenteric lymph nodes, spleen, and liver.

of cell trapping seen in spleen (Fig. 4) after intravenous antigen injection. In the case of subcutaneous SE administration, spleen and liver localization are parallel in stimulated and normal groups of recipients. As cells leave the spleen between 1 and 24 hr they may be preferentially trapped in the draining lymph nodes at the expense of cell distribution to the right and mesenteric nodes. The dynamics involve mainly the distribution of cells to lymph node between 12 and 48 hr, whereas after intravenous injection of SE, the dynamics involve mainly the distribution of cells between lymph node and spleen during the first 24 hr.

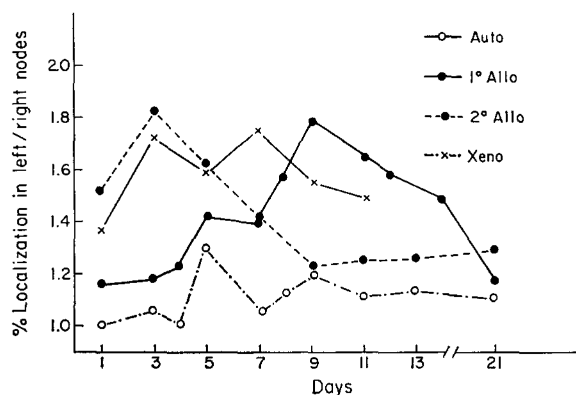

FIG. 6. Comparison of labeled cell localization in the right and left nodes of skin-grafted mice. Labeled lymph node cells were injected into groups of three to nine mice grafted on the left thorax with (*a*) CBA/J skin ---O--- (auto); (*b*) C57BL/6J skin —●— (1° allo); (*c*) C57BL/6J skin twice 3 wk apart ---•--- (2° allo); or (*d*) Lewis rat skin —X— (xeno). Labeled cells were injected 1–21 days after the last graft application, and the mean per cent of localization was determined in recipients' right and left peripheral nodes 20–24 hr later. Results were expressed as a ratio of localization in the left: right nodes.

Effect of Skin Grafting on the Distribution of Labeled Lymphocytes.—Labeled lymphocytes were injected into mice 1–21 days after the grafting of rat, C57BL/6J, or CBA/J (control) skin onto the left thorax. The localization of labeled lymphocytes was compared in the right and left peripheral nodes after 24 hr, and results were expressed as a ratio of localization in the left: right nodes (Fig. 6). The ratio of cell localization in control mice remained close to 1. However, in mice undergoing a first set rejection of C57BL/6J skin, elevated localization of labeled cells occurred in the left nodes, with a maximal ratio of approximately 1.8 at 9 days after grafting. In mice undergoing a second-set allograft rejection, maximal trapping of lymphocytes in the draining nodes occurred at 3 days. The early response to a first set xenograft closely approximated that found for a second-set allograft, i.e., maximal trapping when labeled cells were injected 3 days after grafting. No differences were observed in localization of labeled cells into mesenteric nodes, spleen, or liver in control and experimental groups.

Effect of Number of Injected Cells on the Trapping of Lymphocytes.—In the preceding experiments antigen-stimulated and control mice routinely received 10^7 labeled lymphocytes. Approximately 5–7 $\times$ 10^5 more cells were trapped in spleens of intravenously antigen-stimulated recipients than in controls. 2–4 $\times$ 10^5 more cells were trapped in draining lymph nodes of subcutaneously antigen-stimulated mice. The relation between the injected cell dose and the number of cells trapped was therefore investigated. Groups of recipients were given 5 $\times$ 10^8 SE intravenously. 1 hr later 10^5–10^8 ^{51}Cr-labeled lymphocytes were administered intravenously to antigen-stimulated and control groups, and the per cent

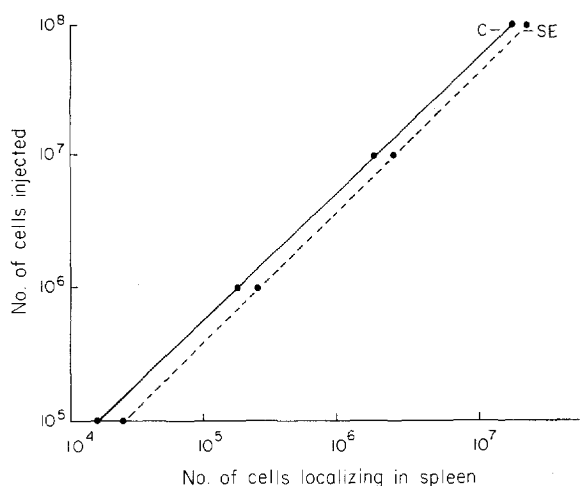

FIG. 7. Effect of number of injected cells on the trapping of lymphocytes. The splenic localization of 10^5, 10^6, 10^7, or 10^8 ^{51}Cr-labeled lymph node cells was determined after 24 hr in groups of three recipients which received either 5 $\times$ 10^8 SE i.v. 1 hr before cell injection ---•---, or no antigen (control) —•—.

of distribution to spleen determined after 20 hr. In Fig. 7, it is clear that the number of cells trapped in the spleen of antigen-stimulated mice is a linear function of the number of cells injected. The difference between cell localization in experimental and control spleen was consistently found to be 6% of the injected cell dose.

DISCUSSION

By using the system of intravenous transfer of ^{51}Cr-labeled lymphocytes into antigen-stimulated syngeneic recipients, it was possible to demonstrate lymphocyte trapping in the spleen after intravenous and intraperitoneal antigen administration, and in draining lymph nodes after subcutaneous antigen administration or challenge with foreign skin. Such trapping was found in mice under-

going both primary and secondary responses, was antigen dose dependent, could be demonstrated within several hours of antigen injection, and was transient, lasting only 24–48 hr. The kinetics of trapping varied with the route of antigenic challenge.

In these experiments the identity of the cell which accumulates in lymphoid organs after antigenic challenge is most likely a member of the long-lived pool of recirculating lymphocytes. Previous work by Lance and Taub (17) and Zatz and Lance (18) has shown that cells which localize in lymph nodes represent a homogeneous population of recirculating lymphocytes, and that during the first 24 hr after cell injection, a dynamic equilibrium exists between spleen and lymph node in the localization of recirculating cells.

In the present experiments, trapping of lymphocytes in spleen occurred at the expense of the lymph node-seeking population, while trapping in draining lymph nodes was at the expense of cell localization in the rest of the lymph node compartment. Results of other studies on lymphocyte traffic after antigen administration reinforce the idea that antigen-induced trapping of recirculating lymphocytes occurs. Ford (15) studied the migration of thoracic duct lymphocytes across the isolated, perfused rat spleen 90 min after the addition of sheep erythrocytes to the perfusate; he found that the cells were delayed in transit across the spleen resulting in a decreased output of recirculating lymphocytes. Hall and Morris (13, 14) have also shown that the output of lymphocytes in the efferent lymph of a stimulated sheep popliteal node is depressed within 30 min of local antigen injection. In both reports this depession of lymphocyte recirculation persisted for several hours. The coincidence of timing in these experimental situations and in those reported here suggests a relation between impaired recirculation of lymphocytes and lymphocyte trapping.

The kinetics of lymphocyte trapping indicate that it is a transient phenomenon which occurs early and may be involved in the initiation of antibody production. Trapping can be demonstrated within 1 hr of intravenous antigen injection and within 24 hr of subcutaneous antigen injection, and reaches a maximum 24 hr after the infusion of labeled cells. The increased accumulation of cells in lymphoid organs during this time may maximize the chance of contact between localized antigen and antigen-sensitive cells. Ford and Gowans (16) have shown that the magnitude of the antibody response initiated in the isolated perfused rat spleen is proportional to the number of recirculating lymphocytes in the afferent perfusate. Hall and Morris (24) have also reported that afferent lymphocytes can restore immunocompetence to the locally irradiated popliteal node of sheep. In the present experiments, lymphocyte trapping in normal and presensitized recipients of SE, H, and KLH occurs within the first 24 hr after antigen administration; the timing of this trap indicates that it is involved in an early feature of the humoral response, probably antigen recognition preliminary to initiation of antibody production.

The tempo of lymphocyte trapping in grafted mice may bear a similar relationship to the initiation of the humoral component of graft rejection. In the first-set allograft response maximal trapping was seen 9 days after grafting, at a time when graft destruction is already well advanced. Breakdown of the graft at this time by sensitized lymphocytes and macrophages would result in transport of antigenic debris to the draining nodes. Gillette and Lance (25) have in fact noted increased accumulations of ^{51}Cr-labeled macrophages at a graft site 9 days after the application of a primary allograft. Similarly, maximal trapping of lymphocytes in the draining nodes 3 days after grafting in the second-set allograft or first-set xenograft response, corresponds to an earlier time of graft rejection, and again precedes peak antibody production by several days (26).

Thus antigen-induced trapping of afferent lymphocytes is probably an important feature in the initiation of humoral immune responses. Dresser et al. (10) have pointed out that adjuvants alone can also cause increased accumulation of lymphocytes in draining nodes; they suggested that this phenomenon could explain in part how adjuvants function to enhance immune responsiveness. While the present experiments indicate that adjuvant is not required for the retention of lymphocytes, it is possible that a greater degree of trapping could be elicited with adjuvant and antigen than with either component alone, resulting in enhancement of antibody production.

When high doses of particulate antigen were administered intravenously, trapping of circulating cells was abolished in spleen, and localization of labeled cells was diminished in liver. One possible explanation is that decreased splenic localization, with concomitant increased lymph node localization, is due to decreased splenic blood flow. Preliminary experiments (MZ, EL, unpublished observations) with Cr-51 labeled mouse erythrocytes injected into mice previously stimulated with high doses of sheep erythrocytes, indicate diminished blood flow may indeed occur; this possibility is under further investigation. Conversely, Dresser, et. al. (10) have shown that trapping of lymphocytes in lymph nodes of mice locally challenged with adjuvant is not due to increased blood flow.

The abolition of increased splenic localization with high doses of antigen may be related to the phenomenon of high zone tolerance. Trapping of lymphocytes appears to be associated with initiation of immune responses; prevention of trapping with high doses may alter the balance of antigen–cell contact in the periphery *versus* in the spleen, resulting in a shift from induction of immunity to induction of tolerance.

Initial increases in cell numbers and organ weight of lymph nodes and spleens of antigenically stimulated animals has been attributed to recruitment of lymphocytes from the circulation (10–12, 27). The experiments reported here indicate that such recruitment may be accomplished by means of an antigen-induced cell trap. In considering the operation of this trap, several questions arise: (*a*) what is the specificity of the trap? (*b*) What is the specificity of the

trapped cells? (*c*) What is the mechanism of action of this trap? We will briefly consider each of these questions.

(*a*) *Specificity of Trap.*—The available evidence indicates that there are both specific and nonspecific elements present. The fact that lymphocyte trapping can be elicited at lower antigen doses in presensitized recipients than in nonsensitized recipients argues for at least partial specificity, since presensitization with an antigen different from the challenging one will not alter the dose requirements for trapping (unpublished observations). The accelerated tempo of trapping which occurs in a secondary allograft response as compared with that of a primary allograft response also indicates a degree of immunological specificity. On the other hand, retention of lymphocytes in antigenically stimulated organs can be demonstrated when cells are injected within 1 hr of intravenous antigen administration; the rapidity of this phenomenon probably precludes specific blast transformation, proliferation, and elaboration of antigen-induced lymphokines as factors contributing to trapping.

(*b*) *Specificity of the Trapped Cells.*—Retention of recirculating lymphocytes after antigenic stimulation could be related to contact of antigen-sensitive cells with antigen contained on dendritic processes of perifollicular or medullary macrophages (28). The finding that lymphocyte trapping can be achieved at lower antigen doses in presensitized recipients would be in keeping with such a view, since localization of antigen on perifollicular macrophages of lymphoid tissues is known to be more efficient in secondary responses (29). The route of recirculation of lymphocytes (30) would bring them into close proximity to such antigen-bearing macrophages. The results of the present experiments, in which it was shown that antigen administration must precede cell injection in order to demonstrate trapping, suggests that some antigen localization is required.

While some of the trapped cells may be involved in specific interactions with antigen, most probably are not. A fairly constant proportion (6%) of injected cells were retained in the antigenically stimulated spleen. However, it is known that only approximately 0.01% of a lymphoid population is specifically antigen sensitive (31) or antigen binding (32, 33). It would seem likely therefore that relatively large numbers of circulating lymphocytes are nonspecifically trapped after antigenic stimulation, thereby possibly facilitating the selective recruitment of smaller numbers of specifically antigen-sensitive cells. This view of lymphocyte trapping is depicted in Fig. 8.

(*c*) *Mechanism of Trapping.*—The actual mechanism by which lymphocyte trapping is accomplished remains unclear. Accumulations of lymphocytes in antigenically stimulated organs could be due to the release of a soluble factor. The rapidity with which trapping can be demonstrated after an antigen injection probably eliminates specifically elaborated lymphokines as mediators (34). However, the release of nonspecific pharmacologically active agents might retard the migration of lymphocytes; Roberts (35) has reported that within

hours of antigenic stimulation mast cells accumulate in the medullary and interfollicular regions of mouse lymph nodes. Release of histamine or related substances could affect the passage of lymphocytes through the lymphoid spaces.

Recent work by De Sousa and Parrott (7) provides a possible morphological basis for the functioning of the cell trap; they have reported that within 24 hr after local application of oxazolone to the skin, plugging of the lymphoid sinuses with clusters of small lymphocytes occurs in the draining nodes of mice.

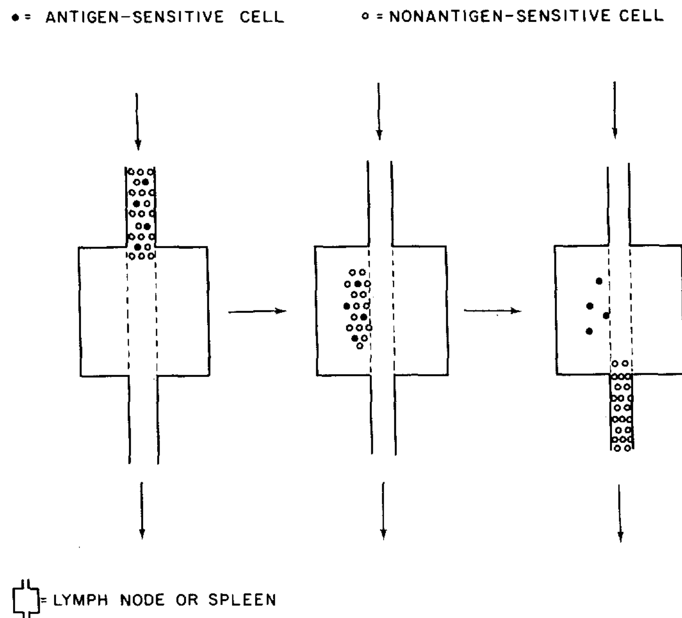

FIG. 8. Lymphocyte trapping. ○ = nonspecific cells, ● = specific antigen-sensitive cells.

Such plugging might well serve to retard the transit of lymph-borne and blood-borne recirculating cells.

Additional experiments are now in progress to further elucidate the precise nature of this cell trap and its relevance to the immune response.

SUMMARY

The localization of syngeneic ^{51}Cr-labeled lymph node cells was investigated in CBA/J mice previously challenged with sheep erythrocytes, *Salmonella H* antigen, keyhole limpet hemocyanin, C57BL/6J skin, or rat skin. The effect of time, dose, and route of antigen administration on lymphocyte migration was studied in both primary and secondary responses.

When the distribution pattern of lymphocytes was examined after 20–24 hr,

it was found that increased localization of labeled cells occurred in spleen after intravenous or intraperitoneal antigen injection, and in draining lymph nodes after subcutaneous antigen injection or skin grafting. Increased localization (trapping) of lymphocytes was antigen dose dependent and could be demonstrated when 1–6 hr had elapsed between intravenous antigen administration, or when 24 hr had elapsed between subcutaneous antigen administration and intravenous cell infusion. Trapping was transient, lasting approximately 24 hr. Maximal trapping of lymphocytes in the draining nodes occurred 9 days after skin grafting in the first-set allograft response, and 3 days after grafting in the second-set allograft and first-set xenograft responses.

The cell type trapped, the specificity and mechanism of action of the trap, and the role of lymphocyte trapping in the initiation of immune responses are discussed.

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