REGIONAL BLOOD FLOW AND ITS RELATIONSHIP TO LYMPHOCYTE AND LYMPHOBLAST TRAFFIC DURING A PRIMARY IMMUNE REACTION*

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A criterial feature of both the initiation and the expression of an immunological response is the contact of an antigen with cells capable of recognizing and interacting with that antigen. An understanding of the mechanisms which facilitate this process is important for an appreciation of the overall physiology of the immune response and its control. Studies in various mammals have shown that there is a pool of small lymphocytes which are capable of continuously recirculating between the blood and lymphoid tissue (1–3). It has been found that during a reaction to various antigens, the number of lymphocytes entering and leaving a lymph node is increased substantially (3–6). Changes in the blood flow to reacting lymph nodes have been described during the primary immune response in rabbits (7, 8), as well as in sheep during secondary immune reactions (8). The observation that increased lymphocyte output in the efferent lymph of sheep lymph nodes occurred at the same time as regional blood flow to the node was increased led to the proposal that vascular changes and increased cellular traffic may be directly related phenomena (8).

During the primary sensitization of skin of mice with a contact sensitizing agent such as oxazolone, an inflammatory reaction in the skin occurs at the same time as an immune reaction is begun in the draining lymph node (9). It is now well recognized that thymus-dependent blast cells, generated in the draining lymph node in response to oxazolone, will accumulate in the ear skin of mice treated with oxazolone (10–12). Among the hallmarks of inflammatory injury are the phenomena of hyperemia, increased vascular permeability, and leukocyte emigration from the blood into the injured area. Although it has been shown that oxazolone-stimulated lymphoblast accumulation in inflamed skin is not related to vascular permeability changes alone (13), we wished to investigate what potential role regional blood flow changes may play in alterations in lymphoblast distribution to skin during oxazolone-induced inflammation and to test the hypothesis that the delivery of lymphocytes to the draining lymph node may be related to changes in regional blood flow to the reacting lymphoid tissue. Changes in both regional blood flow to oxazolone-painted skin and the draining lymph nodes and the localization of labeled lymphoblasts and lymphocytes have been studied in the same animal. We find that hyperemia in the inflamed

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skin is correlated with increased lymphoblast accumulation in the affected area, that the draining lymph nodes experience an increase in regional blood flow in proportion to their proliferating mass, and that this increased blood flow to the reacting nodes is associated with increased localization of small lymphocytes within the node.

Materials and Methods

Mice. 7- to 14-wk-old mice of inbred National Institutes of Health strain maintained in this department were used. Mice of the same age, sex, and strain were used for each experiment.

Choice of Method for Assessing Cardiac Output Distribution. Two methods are available for measuring regional blood flow distribution, i.e. ^{86}RbCl and radiolabeled microspheres. Studies, in which both techniques have been used concurrently, have shown a good agreement between the measures obtained for most tissues (14) as well as for lymph nodes in particular (7). In our studies, the combination of the high energy of γ-emission of rubidium, along with its more simple requirements for vascular access, made rubidium advantageous for the study of large number of animals which had already received labeled cell innocula. This allowed for an experimental design in which relationships between cell distribution and regional blood flow could be directly tested.

Measurement of the Cardiac Output Distribution (% C.O.).[1] C.O. distribution was measured by a modification of the method originally described by Sapirstein using RbCl (15). Under light ether anaesthesia an intravenous cannula was inserted into a lateral tail vein of the mouse under study. A volume of 0.9% saline was injected to ensure patency followed by injection of a known volume of ^{86}RbCl diluted with 0.9% saline. This was immediately flushed through with an equal volume of 0.9% saline. After 45 s, the animal was killed by injecting saturated KCl via the same cannula, the abdomen was opened, and the aorta and vena cava were cut to allow exsanguination of the animal. The tissues of interest were then dissected out and placed in plastic containers for counting.

Choice of Cell Suspensions. We have chosen to study the distribution of oxazolone-induced [^{125}I]UdR-labeled lymphoblasts in oxazolone-sensitized mice, as this is now a well-characterized system (10–12), as well as the distribution of ^{51}Cr-labeled lymphocytes prepared from mesenteric and inguinal lymph nodes of unstimulated donors. We feel justified in using this mixture of cells as a representation of the cells available for recirculation because it contains a high proportion of small cells (16), and the few lymphoblasts which may be present will be largely of mesenteric origin with little capacity to extravasate into a peripheral site (17, 18).

Cell Suspensions. Mesenteric lymph node (MLN) or inguinal lymph node (IN) cells from untreated animals or stimulated peripheral lymph node cells from picryl chloride-stimulated animals (PC-PLN) or 2-ethoxy-4-methyl oxazolone (oxazolone)-stimulated animals (OX-PLN) were prepared at room temperature by gentle teasing of the lymph nodes in RPMI-1640 medium (Gibco Bio-Cult Ltd., Glasgow, Scotland) containing 5% fetal calf serum (FCS) (Gibco Bio-Cult Ltd.). The cell suspensions were then filtered through a 2-cm column of glass wool to remove debris and fatty material. OX-PLN or PC-PLN cells were prepared from the brachial and axillary nodes of animals which had been painted with 10-mg of oxazolone of picryl chloride on the shaved flanks and shoulders 3 d before cell preparation. The number of viable cells in suspension was determined by their ability to exclude 0.2% eosin.

In Vitro Labeling of Cells. To label blast cells undergoing DNA synthesis, MLN or OX-PLN cells were incubated with the thymidine analogue [^{125}I]5-iodo-2-deoxyuridine ([I]UDR) obtained from the Radiochemical Centre, Amersham, England. Incubations were carried out in RPMI-1640 with 5% FCS containing 0.5 μCi/10^7 cells/ml for 60 min at 37°C in a shaking water bath. To label small lymphocytes as well as blast cells, MLN and combined MLN and IN cell suspensions were incubated with (^{51}Cr) sodium chromate (Radiochemical Centre). Incubations were carried out at a concentration of 50 μCi/10^8 cells/ml of RPMI-1640 with

[1] *Abbreviations used in this paper*: AN, auricular nodes; bg, background; C. O., cardiac output; FCS, fetal calf serum; IN, inguinal lymph node; [I]UdR, [^{125}I]5-iodo-2-deoxyuridine; MLN, mesenteric lymph node; OX-PLN, oxazolone-stimulated peripheral lymph node(s); PC-PLN, picrylchloride-stimulated peripheral lymph node(s).

FCS at 37°C for 30 min. After each form of labeling, cells were washed three times in medium alone (RPMI-1640 without FCS). Injection doses of 1.5–2 × 10^7 cells for MLN, blasts, 1 × 10^7 cells for OX-PLN, and 5 × 10^6 cells for ^{51}Cr-labeled cells were given through a lateral tail vein in a 0.4-ml volume. Multiple samples of each injection dose were retained for counting of the administered radioactivity.

Treatment of Recipients. Mice were either left untreated or sensitised with 10 mg of oxazolone or picryl chloride topically on the ears at various times before cell transfer. 24 h after cell transfer the animals were injected with ^{86}RbCl for estimation of cardiac output distribution before killing. In those experiments where cell distribution was assessed alone, the animal was subjected to cannulation and injection as before except that an injection of saline replaced the dose of ^{86}RbCl and the animal was killed by exsanguination.

Counting Methods. The radioactivity in the tissues studied was measured by counting in a gamma counter (ICN-Tracer Lab Instruments, Gamma Set 500) with preset scalars for ^{125}I and ^{51}Cr and an adjustable scalar which could be set for measurement of ^{86}Rb emissions. The peak energy of γ-emissions for the isotopes ^{125}I, ^{51}Cr, and ^{86}Rb are, 0.035, 0.32, and 1.1 MeV, respectively (19), and therefore adjustment of the variable scalar to high thresholds allowed resolution of ^{86}Rb emission from that of either ^{125}I or ^{51}Cr. In the double-label experiments, simultaneous counts were performed on two channels measuring either of ^{125}I or ^{51}Cr and ^{86}Rb. Under these circumstances, ^{86}Rb could be measured independently of the cell-associated isotopes but there was overlap of ^{86}Rb counts into the lower energy scalars. Within each experiment, the overlap of the counts of ^{86}Rb measured on the adjustable scalar compared with that on the fixed scalar of the other isotope was determined. Background (bg) counts were measured for each scalar within each experiment.

The percentage of the cardiac output (% C.O.) going to a particular organ was expressed as the total ^{86}Rb activity which was found in that tissue as a percentage of the total ^{86}Rb activity injected with correction for background as shown in equation 1.

$$\%\ \text{C. O.}_i = \left(\frac{^{86}\text{Rb counts} - \text{bg}}{^{86}\text{Rb injected}}\right) \times 100 \quad \text{(equation 1)}.$$

The in vivo distribution of labeled cells within a given tissue was expressed as a percentage of the injected dose of cells by calculation from the simultaneous measurements on a scalar (scalar 1) preset for counting a cell-label isotope (^{125}I or ^{51}Cr) and the scalar adjusted to resolve ^{86}Rb emission (scalar 2) as shown in equation 2.

$$(\%\ \text{cell-label injected})_i = \frac{(\text{scalar 1 counts} - \text{bg}_1) - \text{overlap ratio} \times (\text{Scalar 2 counts} - \text{bg}_2)}{(\text{scalar 1 counts of injected dose} - \text{bg}_1)} \times 100 \quad \text{(equation 2)}.$$

Statistical Analysis. The significance of differences in means of various groups was assessed using Student's *t* test. Correlation coefficients (*r*) were calculated by standard methods (20, 21).

The significance level of correlation coefficients (i.e. the probability whether the observed correlation coefficient could have arisen by chance) was assessed by Student's *t* test using the form

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

with $n - 2$, degrees of freedom (21).

Results

The Distribution of ^{86}RbCl Activity in Normal Mice. That portion of an intravenous injection dose of an indicator such as ^{86}RbCl which is found in a particular organ or tissue within 1 min of the injection is a measure of the fractional distribution of the C.O. which is received by that tissue (15, 22). The distribution of ^{86}RbCl expressed as

TABLE I

The Fractional Distribution of C.O. Determined by ^{86}RbCl Method in Normal Mice (n = 8)

Organ	C.O. ± S.D.	C.O./g
	%	%
IN	0.10 ± 0.02	3.52 ± 0.66
MLN	0.49 ± 0.25	3.59 ± 0.91
Spleen	0.36 ± 0.08	3.78 ± 0.93
Small intestine	17.7 ± 2.6	8.2 ± 1.1
Large intestine*	6.8 ± 0.9	5.9 ± 1.0
Heart	2.5 ± 0.5	27.3 ± 3.9
L. kidney	5.6 ± 1.6	30.5 ± 9.7
R. kidney	5.0 ± 1.4	28.8 ± 8.7

* Including cecum.

TABLE II

24-h Distribution of Labeled Cells Measured With or Without ^{86}RbCl Injection

Cell inoculum	Method of measurement	Number of Mice	Mean Percent injected dose of cells ± SD			
			IN	MLN	SI	Spleen
			%			
[^{125}I]UdR-MLN	Single label	5	0.20 ± 0.10	0.38 ± 0.05	4.34 ± 0.40	0.64 ± 0.34
[^{125}I]UdR-MLN	Double label	5	0.13 ± 0.17	0.45 ± 0.15	3.85 ± 0.57	0.54 ± 0.17
^{51}Cr-MLN	Single	5	1.38 ± 0.36	6.24 ± 0.78	1.96 ± 0.29	8.69 ± 0.93
^{51}Cr-MLN	Double	5	1.80 ± 0.30	7.02 ± 0.95	2.02 ± 0.31	9.19 ± 0.56

a percentage of the C.O. for normal mice is shown in Table I. The perfusion of organs such as kidney and heart expressed relative to their mass is much higher than tissues like the bowel even though the percentage of the C.O. which goes to renal or cardiac tissues is only of the order of 5% or less. By contrast, although different lymphoid tissues, such as inguinal and mesenteric nodes and spleen receive different fractions of the C.O., their perfusion per gram is very similar and substantially less than highly perfused tissues such as kidney (Table I).

Concomitant Measurements of Cell Distribution and Fractional C.O. To assess the validity of measuring the distribution of both labeled cells and the ^{86}RbCl activity in the same animal, experiments were performed in which the distribution of cells labeled with either [^{125}I]UdR or ^{51}Cr was measured with or without the injection of ^{86}RbCl at the time of sacrifice. The results (Table II) show no significant differences in the distribution of cell labels in either lymphoid or nonlymphoid tissue when rubidium was used as compared with the situation when no second label was introduced into the animal. This substantiates our expectation that the different emissions of cell labels and the ^{86}Rb used for monitoring of the C.O. fractionation can be successfully resolved and that estimates of cell localization as measured by the double-label technique, are not affected by the presence of the second label.

The Effect of Oxazolone on the Regional Blood Flow and Weight of the Auricular Node. The changes in wet weight and the fraction of the C.O. received by auricular lymph nodes with and without oxazolone applied to the ears is shown in Table III. The weight of the auricular nodes draining oxazolone-painted ears is significantly increased within

TABLE III

The Effect of Oxazolone on the Regional Blood Flow and Wet Weight of Auricular Lymph Nodes‡*

Treatment schedule of oxazolone recipients	C.O.	Weight
	%	*mg*
No sensitization	0.09 ± 0.05	15 ± 3
1 d before	0.26 ± 0.07§	27 ± 4§
2 d before	0.42 ± 0.08‖	38 ± 3‖
3 d before	0.48 ± 12‖	64 ± 16‖

* 10 mg oxazolone was painted on the ears at the times shown.
‡ Responses are the mean ± SD of four animals per group.
§ $P < 0.01$ significantly different from nonsensitized recipient.
‖ $P < 0.001$ significantly different from nonsensitized recipient.

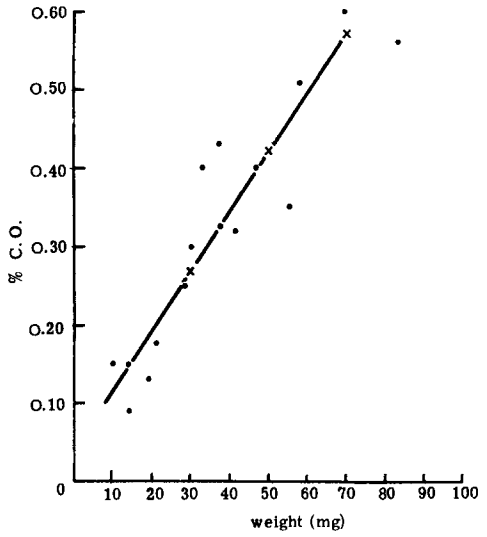

FIG. 1. The relationship of regional blood flow (%C.O.) received by auricular lymph nodes to their mass during primary reaction to oxazolone. The regression line is drawn through points (X) calculated from the analysis of the observed data. The correlation coefficient for the % C.O. received vs. weight is $r = 0.86$ with a significance value $P < 0.001$.

1 d of oxazolone treatment as is the percentage of the C.O. received by the nodes. As the inflammation induced by oxazolone proceeds over 3 d, there is a greater than fourfold increase in both the perfusion of the auricular nodes and their mass, indeed, there is a direct correlation between weight and blood flow to the auricular nodes of individual animals (Fig. 1).

Cell Localization and Regional Blood Flow to Inflamed Ear and Draining Node during Response to Oxazolone. To assess the relationship between blood flow alterations and cell distribution during the primary response to oxazolone we have studied the in vivo localization of labeled cells and the cardiac output distribution concomitantly in animals which have been treated with oxazolone. Animals for study were either unsensitized or treated with oxazolone painting to the ears at various times (0–3 d) before cell transfer. 24 h after cell transfer, the regional blood flow of different tissues

TABLE IV

The Effect of Oxazolone on the Regional Blood Flow and 24-h Localization of [^{125}I]UdR-labeled OX-PLN to the Ear and Auricular Lymph Nodes of Mice Undergoing Sensitization at Different Times before Cell Transfer‡*

Treatment schedule of oxazolone recipients	C.O.		Injected dose of cells	
		%		%
No sensitization	Ears	0.31 ± 0.09	Ears	0.06 ± 0.04
	AN	0.18 ± 0.08	AN	0.09 ± 0.04
Day of cell transfer	Ears	0.49 ± 0.11§	Ears	0.66 ± 0.19‖
	AN	0.35 ± 0.21	AN	0.59 ± 0.29¶
1 d before cell transfer	Ears	0.48 ± 0.13§	Ears	1.54 ± 0.75‖
	AN	0.41 ± 0.09‖	AN	2.39 ± 0.38‖
2 d before cell transfer	Ears	0.52 ± 0.14§	Ears	0.87 ± 0.22‖
	AN	0.36 ± 0.08¶	AN	1.27 ± 0.22‖
3 d before cell transfer	Ears	0.46 ± 0.09§	Ears	1.50 ± 0.07‖
	AN	0.32 ± 0.18	AN	0.59 ± 0.37

* 10 mg of oxazolone was painted on the ears at times shown.
‡ Responses shown are the means ± SD for five to six mice per group.
§ $P < 0.05$ significantly different from value for organ in animals not treated with oxazolone.
‖ $p < 0.001$.
¶ $p < 0.01$.

was studied using the ^{86}RbCl method, at which time the animals were sacrificed. The distribution of [^{125}I]UdR-labeled blast cells (OX-PLN) and regional blood flow to ears and the auricular nodes of animals at various stages in their response to oxazolone painting is shown in Table IV. There is a maximal enhancement of both the blood flow and blast localization in the auricular nodes when oxazolone painting occurred between 1 and 2 d before the cell transfer with a subsequent decrease in blood flow and blast localization after this time.

The fraction of C.O. which goes to oxazolone-treated ears reaches a maximum by 1 d after painting and persists throughout the time period studied although the localization of blast cells is maximal by 48 h after oxazolone treatment and does not significantly decrease thereafter. The results of a similar experiment in which the localization of ^{51}Cr-labeled cells from MLN and inguinal nodes of untreated donors were studied during oxazolone treatment are presented in Table V. This shows that the blood flow response in the auricular nodes (AN) and cell localization in the AN is very similar in pattern to the blast cell experiment. The accumulation of ^{51}Cr-labeled cells in the ears is however much smaller than it is for OX-PLN blast cells.

In these experiments, both cell distribution and regional blood flow were studied in each individual animal. To explore the relationship between blood flow and cell localization the paired observations of the fraction of C.O. and the 24-h localizations of different cells received by either the inflamed ear or the draining auricular node were subjected to correlation analysis. The results of these analyses are presented in Table VII. These results demonstrate that for [^{125}I]UdR-labeled OX-PLN cells there is a significant correlation of the 24-h localization of these cells with the measured regional blood flow to the ears throughout various stages of the sensitization process but that the localization of [^{125}I]UdR OX-PLN within auricular nodes did not show significant correlation with the blood flow to that organ. The 24-h localization of ^{51}Cr

TABLE V

The Effect of Oxazolone‡ on Regional Blood Flow and 24-h Localization of ^{51}Cr-Labeled Cells§ to Ears and Auricular Nodes of Mice Undergoing Sensitization at Different Times*

Treatment schedule of oxazolone recipients	C.O. to that organ		Injected dose of cells found in that organ	
		%		%
No sensitization	Ears	0.33 ± 0.14	Ears	0.04 ± 0.02
	AN	0.05 ± 0.10	AN	0.71 ± 0.30
Day of cell transfer	Ears	0.54 ± 0.10‡	Ears	0.08 ± 0.03‡
	AN	0.12 ± 0.04	AN	1.29 ± 0.54
1 d before cell transfer	Ears	0.60 ± 0.22‡	Ears	0.11 ± 0.05‡
	AN	0.33 ± 0.15‖	AN	2.40 ± 0.87‖
2 d before cell transfer	Ears	0.70 ± 0.08‖	Ears	0.09 ± 0.04
	AN	0.35 ± 0.08‖	AN	2.27 ± 0.55¶
3 d before cell transfer	Ears	0.79 ± 0.27‡	Ears	0.12 ± 0.04‡
	AN	0.24 ± 0.12‡	AN	2.17 ± 0.71‖

* 10 mg of oxazolone was painted on the ears at times shown.
§ Responses shown are the means ± SD for five to six animals per group.
‡ $P < 0.05$ significantly different from value for that organ in animals not treated with oxazolone.
‖ $P < 0.01$.
¶ $P < 0.001$.

TABLE VI

*The 24-h Localization of Cells and Regional Blood Flow to Inguinal and Mesenteric Nodes from Animals in Various Stages of Response to Oxazolone**

	IN	MLN
	%	
Injected [^{125}I]UdR OX PLN (n = 27 animals)	0.28 ± 0.12	0.19 ± 0.06
C.O.	0.13 ± 0.05	0.54 ± 0.12
Injected ^{51}Cr cells (n = 29 animals)	1.61 ± 0.47	7.53 ± 1.90
C.O.	0.12 ± 0.09	0.53 ± 0.19

* Measures are mean ± SD.

cells from the MLN and IN of nonsensitized donors, on the other hand, showed no significant correlation with blood flow within the ears but a significant correlation was found for the localization of these cells within the draining auricular nodes.

The 24-h localization of both [^{125}I]UdR OX-PLN and ^{51}Cr-labeled cells, as well as regional blood flow, was also measured for both IN and MLN tissue from each of the animals in the experiments described in Tables IV and V and the overall mean responses for these nodes are presented in Table VI. The results of the correlation analysis of each type of cell localization with the regional blood flow for different lymph nodes is also presented in Table VII. The localization of [^{125}I]UdR OX-PLN in auricular, inguinal and, mesenteric nodes showed no significant correlation with blood flow to that organ, but there was a significant positive relationship between the localization of ^{51}Cr-labeled cells and the regional blood flow to these same lymph nodes (Fig. 2).

Because the only site in which we observed a correlation between blast cells and

TABLE VII

Results of Correlation Analysis of Cell Localization with Fractional Blood Flow for Different Tissues during Primary Sensitization with Oxazolone

Tissue	[^{125}I]UdR OX-PLN	^{51}Cr MLN + IN
Ears	$r = 0.50$	$r = 0.13$
	$n = 27$	$n = 26$
	$P < 0.01$	P = NS
Auricular nodes	$r = 0.17$	$r = 0.55$
	$n = 27$	$n = 26$
	P = NS	$P < 0.01$
Auricular, inguinal and mesenteric nodes combined	$r = 0.19$	$r = 0.85$
	$n = 72$	$n = 72$
	P = NS	$P < 0.001$

r, correlation coefficient; n, number of paired observations analysed; P, probability that the correlation coefficient is a result of chance; NS, not significant.

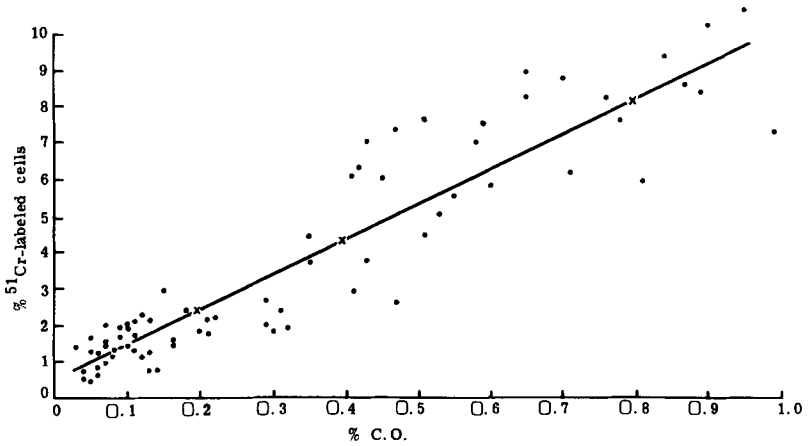

FIG. 2. The relationship of the 24-h localization of ^{51}Cr-labeled lymphocytes to the regional blood flow of auricular, inguinal, and mesenteric lymph nodes. The regression line was drawn through the points (X) obtained from the analysis of the observed data.

blood flow was in ear skin, the question arises as to whether this correlation is antigen dependent. Therefore, we have compared the distribution of [^{125}I]UdR OX-PLN in the ears of animals treated with either oxazolone or a noncross-reacting contact sensitizer, picryl chloride (Table VIII). This demonstrates that there is increased localization of OX-PLN into the inflamed ear regardless of the agent applied which parallels the degree of hyperemia that has developed. At each stage of the sensitization, however, there is a small but statistically significant preference for increased localization of OX-PLN in oxazolone-induced lesions. This tendency for some degree of antigen preference is also demonstrated when the localization of oxazolone- and picryl chloride-stimulated blast cells is compared in oxazolone-treated animals (Table IX).

Although the localization of both types of cells is enhanced during the reaction to oxazolone, there is a preferential enhancement of OX-PLN into oxazolone-treated ears. The analysis of both blast cell distribution and regional blood flow in the ears of animals responding to oxazolone or picryl chloride, (Table X), shows that there is a significant correlation between cell localization and blood flow regardless of the agent of the inflammation.

TABLE VIII

The Effect of Oxazolone or Picryl Chloride on the Regional Blood Flow and 24-h Localization of [^{125}I]UdR OX-PLN to the Ears‡*

Treatment of recipients	C.O.	Injected dose of cells
	%	%
Nonsensitized	0.27 ± 0.08	0.04 ± 0.01
Picryl chloride on day of cell transfer	0.64 ± 0.11	0.68 ± 0.14§
Oxazolone on day of cell transfer	0.77 ± 0.05	1.00 ± 0.14§
Picryl chloride 1 d before cell transfer	0.74 ± 0.07	1.17 ± 0.35§
Oxazolone 1 d before cell transfer	0.68 ± 0.23	1.92 ± 0.23§
Picryl chloride 2 d before cell transfer	0.97 ± 0.29	1.12 ± 0.19§
Oxazolone 2 d before cell transfer	0.97 ± 0.31	1.93 ± 0.45§

* 10 mg of either oxazolone or picryl chloride was painted on the ears at times shown.
‡ Responses are the mean ± SD for five to six mice per group.
§ $P < 0.01$ significantly different response for comparison of the value in animals treated with picryl chloride vs. oxazolone at the same time.

TABLE IX

The Effect of Oxazolone on the Regional Blood Flow and 24-h Localization of [^{125}I]UdR-Labeled OX-PLN or PC-PLN to the Ears‡*

Treatment of recipients	Donor cells	C.O.	Injected dose of cells
		%	%
Unsensitized	PC-PLN	0.29 ± 0.07	0.03 ± 0.01
Unsensitized	OX-PLN	0.34 ± 0.06	0.05 ± 0.01
Oxazolone on day of cell transfer	PC-PLN	0.64 ± 0.20	0.54 ± 0.11§
Oxazolone on day of cell transfer	OX-PLN	0.55 ± 0.10	1.49 ± 0.30§
Oxazolone 1 d before cell transfer	PC-PLN	0.49 ± 0.09	0.66 ± 0.12§
Oxazolone 1 d before cell transfer	OX-PLN	0.60 ± 0.18	1.34 ± 0.46§

* 10 mg of oxazolone was painted on the ears at times shown.
‡ Responses are the mean ± SD for five to six mice per group.
§ $P < 0.01$ Significantly different response for the value found in the ears of animals receiving OX-PLN vs. PC-PLN at the same stage of oxazolone treatment.

TABLE X

Results of Correlation Analysis of [^{125}I] UdR OX-PLN Localization and Regional Blood Flow to the Ears of Animals Undergoing 1-s Sensitization with Either Oxazolone or Picryl Chloride

Oxazolone	Picryl chloride
$r = 0.58$	$r = 0.62$
$n = 24$	$n = 23$
$P < 0.01$	$P < 0.01$

r, correlation coefficient; n, number of paired observations analysed; P, probability that the correlation coefficient is a result of chance.

Discussion

The purpose of this study was to examine the nature of regional blood flow to the ear and its draining lymph node during a primary immune response in mice and to test the hypothesis that changes in lymphocyte traffic during an immune reaction are directly related to alterations in blood flow to lymphoid tissue (8).

The profile of distribution of the cardiac output for mice (Table I) is in good agreement with that found for other species (8, 14, 15) and the specific perfusion represented as the percent cardiac output received per gram of tissue shows the richness of perfusion expected for tissues such as the heart and kidneys compared with lymphoid tissue. The progressive increase in regional blood flow to auricular nodes draining an area of primary sensitization with oxazolone (Table I) and its correlation with the increase in weight of the nodes (Fig. 1) suggest that a lymph node expands its blood supply and its mass during antigenic stimulation in a connected manner. Our observations of these mass-related blood flow changes in the lymph nodes of mice responding to oxazolone are directly analogous to those of Herman and his colleagues (7) in rabbit lymph nodes responding to primary sensitization with protein antigens. This suggests that an increase in the perfusion of a lymph node undergoing antigenic challenge is a general phenomena of many different species and a fundamental property of lymphoid tissue.

To test the hypothesis that these changes in regional blood flow are related to changes in cell traffic, experiments were performed in which the localization of labeled cells and the regional blood flow were measured in individual animals at various stages of the primary sensitization to oxazolone. We have chosen to measure the cell localization at 24 h, because at this time the distribution of ^{51}Cr-labeled cells will have reached equilibrium with the various lymphocyte pools of the body (23, 5, 6) and the accumulation of oxazolone-stimulated lymphoblasts in inflamed ears is maximal (13).

The application of oxazolone to the ear skin of previously unsensitized animals produces an inflammatory reaction (9) with increased vascular permeability (13) and the accumulation over time of oxazolone-stimulated immunoblasts (11), although these two phenomena are not directly related. In this study we find that the 24-h accumulation of [^{125}I]UdR OX-PLN blasts in skin at various stages of the inflammatory process is positively correlated with the magnitude of the regional blood flow although the more modest 24-h localization of ^{51}Cr-labeled cells is not (Table VII).

The traffic of cells through most nonlymphoid tissues, although up to an order of magnitude less than the flux of cells through the regional lymph node, is a potentially important feature of lymphocyte monitoring of the organism (24). It has been shown in sheep, that the application of a sensitizing agent, such as fluorodinitrobenzene, to the skin can stimulate an increase in the volume, protein concentration, and cell content of afferent lymph draining to the regional lymph node (4). The increased cellularity is largely a result of increases in polymorphic neutrophils and/or macrophages with only modest increases in the small lymphocyte content. Similar observations have been found with subcutaneous injection of other antigens in sheep and rabbits (5, 25).

Our observation of only a modest increase in ^{51}Cr-labeled nonsensitised lymphocytes localisation along with a more substantial increase in the accumulation of [^{125}I]UdR OX-PLN in inflamed ears is in good agreement with those of Asherson and his coworkers (11), and suggests that the lymphoblasts do indeed have a greater propensity to extravasate into inflamed tissue than small lymphocytes.

It has been shown that OX-PLN lymphoblasts will migrate to sites of inflammation besides inflamed skin (17) and it may be that such peripherally stimulated lymphoblasts have a particular affinity to exit from the vascular compartment when presented

with an altered endothelium. This exit could be facilitated if such lymphoblasts can respond to chemotactic stimuli in vivo as well as in vitro (26), further facilitation might be expected from increased delivery resulting from the increased blood flow to the injured area.

Our experiments on the localization of antigen-stimulated lymphoblasts into ears inflamed by a nonrelated antigen suggest that the bulk of the accumulation of those cells is not antigen dependent but related to increased delivery of the cells from the blood. The preferential accumulation of specifically primed blasts within the antigen-related lesions has been previously reported (17) and may represent either the superposition of a specific reaction upon the acute inflammation or minor differences in the course of the reaction elicited by different agents. Allwood (27) has demonstrated that the injection of specifically primed lymph node cells is able to transfer passively contact sensitivity to the agent which primed the donor cells.

The delivery of small lymphocytes to the inflamed skin is also potentially enhanced by the hyperemia to the injured area. The more modest increase of small lymphocytes in the inflamed ear and the lack of a linear relationship with blood flow suggest that the interaction of small lymphocytes with endothelium even in an area of inflammation may be quite different than that of lymphoblasts. Smith and his co-workers (28) found that a chronic granulomatous lesion in sheep was associated with a substantial traffic of lymphocytes from the circulation to afferent lymph and that many of the postcapillary venules in the reactive edge of the lesion were lined with high endothelial cells. This elaboration of high endothelium is analogous to the specialized postcapillary venules of lymph nodes (29, 30) and suggests a special requirement for vascular egress on the part of small lymphocytes. Whatever the detailed requirements are for a particular type of cell to have an increased probability to leave the vascular compartment into an area of inflammation, we conclude that the endothelium-lymphocytic interaction is quite different for large blastic cells as compared with small recirculating lymphocytes.

This difference in propensity to leave the blood stream is also suggested by our observations in lymph nodes. The localization of [^{125}I]UdR labeled OX-PLN cells in the auricular nodes during the primary response to oxazolone is not correlated with the increased blood flow to the node (Table VII) whereas the distribution of ^{51}Cr-labeled lymphocytes measured 24 h after inoculation shows a positive relationship with the degree of perfusion of the lymph nodes. This suggests that requirements for entry into the node may also be quite different for these different cell types. It is unlikely that a direct linear relationship between blast accumulation within auricular lymph nodes and blood flow is being obscured by blasts entering the node via the afferent lymph from the ears because the fall off of OX-PLN cell label from oxazolone-treated ears is slower than the loss of this label from the draining nodes (17).

The correlation between the localization of ^{51}Cr-labeled lymphocytes and fractional blood flow (Table VII) within different lymph nodes suggests that the probability that a particular lymphocyte can be found at equilibrium within a given lymph node is directly related to the degree of perfusion of that lymph node. Hay and Hobbs (8) observed simultaneous increases in blood flow to a reacting lymph node and the cell output of the efferent lymph and suggested that the increased lymphocyte output was directly related to the increased blood flow. Our observations support and extend this hypothesis. We conclude that alterations in regional blood flow can be an important

modulator of both the affector and effector aspects of an immune response. The hyperemic response of inflamed skin induced by oxazolone is related to the increased accumulation of effector lymphoblasts within the affected tissue. The expansion of regional blood flow to a reacting lymph node, on the other hand, can be viewed as a physiological mechanism which facilitates the increased entry of lymphocytes into that node and thereby increases the probability that all lymphocytes that are capable of recognizing an invading antigen will have an opportunity of coming into contact with that antigen.

Summary

The relationship of alterations in blood flow with changes in cell distribution has been studied in an inflammatory site and its draining lymph node during the induction of an immune reaction with oxazolone in mice. The cells which move to the site of inflammation are predominantly lymphoblasts and their increased localization in the inflamed ear is significantly correlated with increased regional blood flow to the inflamed tissue. The existence of this correlation is not antigen dependent although there is a relative increment of lymphoblasts which are specifically primed to the inflammatory agent.

The localization of nonblastic (small) ^{51}Cr-labeled lymphocytes on the other hand is substantial only in lymphoid tissue and during the induction of an immune reaction after oxazolone application, the increase in localization of these cells in the draining lymph node is positively correlated with increased blood flow to the node. Furthermore, the probability of finding ^{51}Cr-labeled lymphocytes in a particular lymph node is related to the regional blood flow which that node receives.

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