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The Cellular Origin of the Lymphocyte Trap

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Summary. We investigated the cellular requirements for lymphocyte trapping. Depletion of lymphocyte populations selectively or indiscriminately did not affect the ability of animals to trap. The variety of materials which initiate trapping was also studied and this information, coupled with the resistance of 'trapping' to severe lymphocyte depletion, is consistent with the hypothesis that the macrophage may be the central cell in initiating the trapping of lymphocytes after antigen stimulation.

INTRODUCTION

The normal kinetics of lymphocyte recirculation may be altered significantly by the administration of immunogen or adjuvant (Hall and Morris, 1965; Ford, 1968; Dresser, Taub and Krantz, 1970; Zatz and Lance, 1971a). Depending upon the route of injection, lymphocytes are sequestered within the draining lymph nodes or the spleen. This net influx of cells has been termed lymphocyte trapping.

Although considerable information exists concerning the effects produced by variations of route, timing and dosage (Zatz and Lance, 1971a), little is known about the biological role of trapping or its precise mechanics. It has been argued that trapping could serve the function of recruiting potential antigen-reactive cells at a site where interaction with antigen would be facilitated (Zatz and Lance, 1971a). Trapping may therefore be a necessary step in the event of immunization.

In this study we investigate the cellular requirements for trapping. Depletion of lymphocyte populations either selectively or indiscriminately did not manifestly interfere with the capacity to trap. On a different tack the range of substances which might or might not elicit trapping was studied. These results taken with the former are consistent with the working hypothesis that the macrophage may be the central cell in springing the lymphocyte trap.

MATERIALS AND METHODS

Experimental design

The method of eliciting and quantifying lymphocyte trapping has been previously reported in detail (Zatz and Lance, 1971a). Briefly, syngeneic lymph node lymphocytes which have been labelled *in vitro* with ^{51}Cr (Bainbridge and Gowland, 1966) are injected intravenously into groups of treated or untreated mice. The localization of these cells is measured in recipient lymph nodes, spleen and liver 24 hours later. The presence of trapping is considered confirmed when the localization with the antigen-stimulated organ is at least 20 per cent greater than that found in the appropriate control. When trapping is measured in the spleen (i.e. after intravenous or intraperitoneal antigen

administration) the relevant controls are animals which received equivalent volumes of phosphate-buffered saline. For lymph node trapping, i.e. after subcutaneous immunization, two sorts of comparisons are available: the lymph nodes on the opposite side of the body, and the lymph nodes of saline-injected controls. Splenic trapping is computed by the following formula:

$$\frac{\text{Experimental} - \text{Control}}{\text{Control}} \times 100.$$

A similar formula is used for lymph node trapping in which the right side nodes serve as controls.

In this model lymph node or splenic trapping is often accompanied by a parallel fall, presumably compensatory, in other sites of localization. This phenomenon has been adequately documented previously (Zatz and Lance, 1971a) and will not be commented upon further in this presentation.

Animals

All experiments were performed on C3H mice of either sex obtained between 8 and 10 weeks of age from the breeding unit at the Clinical Research Centre (Harrow, Middlesex) or a commercial supplier (Scientific Agribusiness Consultants Ltd, Braintree, Essex).

Deprived mice (Davies, Leuchars, Wallis and Koller, 1966) (hereinafter referred to as 'B' mice) were thymectomized at 3 weeks of age, exposed to 900 rad whole body irradiation 2 weeks later and reconstituted with 6–8 million syngeneic bone marrow cells.

Antigen injection

Materials used to provoke trapping were injected via the tail vein or subcutaneously in the left flank. Radiolabelled cells were injected 1 hour after intravenous antigen and 24 hours after subcutaneous antigen. The various materials were always injected in a volume of 0·25 ml and included 5×10^8 sheep erythrocytes (Burroughs Wellcome); 750 μg keyhole limpet haemocyanin (Calbiochem); *Salmonella* 'H' antigen, agglutinable suspension (Burroughs Wellcome); 5 mg bovine serum albumin; 10–1000 μg bovine gamma globulin (Armour Pharmaceutical Co., Eastbourne, England); *Corynebacterium parvum* (Burroughs Wellcome); Freund's complete adjuvant (Gibco); 10 mg vitamin A alcohol (Koch Light Pharmaceuticals); 5–20 mg Carrageenan (Seakem, Bar Harbor, Maine); 2 mg Silica, Dorentrup Quartz number 12, <5 μM (kindly provided by Dr A. Allison); 4 mg carbon particles (Pelican Ink, C11/1431); and latex particles (Difco).

In some experiments presensitization to bovine gamma globulin (BGG) was achieved by the injection of 200 μg BGG emulsified in Freund's complete adjuvant and injected by the intraperitoneal or subcutaneous route. Presensitization was performed 10 days prior to antigen administration.

Lymphocyte depletion

A variety of immunosuppressive manoeuvres were used to achieve selective or indiscriminate lymphocyte depletion.

(a) *Irradiation.* Animals in individual lucite containers received between 400 and 1500 rad of whole body irradiation from a cobalt source (Gamma beam 650) housed at the National Institute for Medical Research at Mill Hill. The target to source distance was 190 cm and irradiation was delivered at 55 rad/min. Under these conditions the LD 50 at 15 days for C3H mice was 1000 rad.

TABLE 1

ANTIGEN-INDUCED LYMPHOCYTE TRAPPING IN C3H MICE*

Mean percentage localization of ^{51}Cr-labelled cells†	Intravenous antigen				Subcutaneous antigen‡				
	Control	SRBC	KLH	*Sal.H.*		Control	SRBC	KLH	*Sal.H.*
Lymph node	17·5±1·2	15·7±1·3	13·3±0·2	11·7±0·8	L	2·7±0·1	4·2±0·2	4·4±0·3	4·0±0·1
					R	2·7±0·3	2·5±0·3	2·2±0·3	2·1±0·2
					M	7·9±0·1	6·5±0·7	6·0±0·8	6·2±0·6
Spleen	16·1±1·0	22·9±0·8	27·3±1·0	27·7±0·1	Spleen	19·6±0·4	19·7±0·6	19·3±1·2	18·6±0·4
Liver	18·7±0·5	17·6±0·4	16·8±1·6	14·5±0·3	Liver	16·2±0·2	15·7±0·7	14·8±1·4	15·4±0·9
Percentage trapping§ (spleen)		42%	70%	72%	Percentage trapping L nodes vs R nodes		68%	100%	94%
Summary of 105 experiments		Mean percentage trapping in spleen after intravenous antigen: 46·6% (range 20%–170%)			Summary of 127 experiments		Mean percentage trapping in draining lymph nodes after subcutaneous antigen: 62·1% (range 20%–210%)		

* Groups of three mice injected with syngeneic ^{51}Cr-labelled lymph node cells after intravenous or subcutaneous antigen.

† Average percentage localization of radiolabelled lymphocytes ± standard deviation is determined by dividing the number of counts localized in an individual organ by the total number of counts injected.

‡ Subcutaneous antigen always injected in left flank.

$$\text{§ The percentage trapping} = \frac{\text{percentage localization in antigen stimulated organ (peripheral lymph node, spleen)} - \text{percentage localization in control organs}}{\text{percentage localization in control organs}}.$$

L = left sided lymph nodes; R = right sided lymph nodes; M = mesenteric lymph nodes.

TABLE 2

EFFECT OF ANTILYMPHOCYTE SERUM ON ANTIGEN-INDUCED LYMPHOCYTE TRAPPING

Mean percentage localization of ^{51}Cr-labelled cells†	Experiment number 110			Experiment number 150		Experiment number 182		
	No antigen	SRBC	KLH	No antigen	SRBC	No antigen	SRBC	KLH
Lymph node	4·7±0·3	5·4±0·5	4·8±0·4	5·6±0·5	7·3±0·3	5·1±0·4	4·8±0·7	4·7±0·2
Spleen	11·4±0·6	17·4±1·3	20·7±0·6	13·3±0·9	20·5±0·8	12·6±0·7	19·4±0·5	21·2±0·9
Liver	30·8±1·4	29·4±1·8	28·1±2·5	30·1±1·8	24·2±1·4	29·8±1·2	27·1±1·3	24·3±1·1
Per cent trapping§		53%	82%		54%		54%	68%

† As in Table 1. § As in Table 1.

Animals in all groups were treated with 1·5 cc of ALS. In addition some received intravenous antigen as indicated prior to receiving syngeneic ^{51}Cr-labelled lymph node cells.

(b) *Antilymphocyte serum.* Rabbit anti-mouse thymocyte serum was prepared by the two pulse method of Levey and Medawar (1966a and b) and assayed as described by Jooste, Lance, Levey, Medawar, Ruszkiewicz, Sharman and Taub (1968). The survival of allogeneic skin grafts in the A to CBA combination was extended to two to three times beyond that of normal controls.

In our experiments 1·5 ml of ALS was given subcutaneously in three divided doses 6, 4 and 2 days prior to antigen administration.

(c) *Cyclophosphamide.* Cyclophosphamide (Endoxana WB Pharmaceuticals) at a dosage of 200–300 mg/kg was injected intraperitoneally on three occasions, 1, 2 and 3 days prior to antigen. This dose was chosen to correspond with findings of Poulter and Turk (1972) who found this regimen to have a maximum effect in the depletion of 'bone marrow-derived' lymphocytes.

(d) *Corticosteroids.* Hydrocortisone acetate (HCA) (Boots Pure Drug Co., Nottingham) was injected intraperitoneally at a dosage of 2·5 mg 2 days prior to antigen. This dose corresponds to that used by Cohen and Claman (1971) and which is known to have significant thymic and peripheral lympholytic effects (Lance and Cooper, 1970).

For all these treatments, groups of six animals were used. Three animals in each group received antigen while the remainder served as controls.

RESULTS

THE EFFECT OF ANTIGEN ADMINISTRATION ON THE DISTRIBUTION OF ^{51}Cr LABELLED CELLS

The results of individual experiments demonstrating trapping either in the spleen after intravenous antigen or in the local lymph nodes after subcutaneous antigen are given in Table 1.

TABLE 3

ANTIGEN-INDUCED LYMPHOCYTE TRAPPING IN 'B' MICE*

Mean percentage localization of ^{51}Cr-labelled cells†	No Antigen	SRBC IV	KLH IV
Lymph Node	17·6±0·4	13·1±0·4	11·4±2·3
Spleen	22·9±1·1	29·6±1·0	28·6±2·8
Liver	15·2±1·8	6·6±1·4	12·0±1·4
Percentage trapping in spleen‡		29%	25%

* All groups represent a mean of three experiments. 'B' mice are animals which had been thymectomized as adults, lethally irradiated and reconstituted with syngeneic bone marrow. Antigen was administered 1 hour prior to syngeneic ^{51}Cr-labelled lymph node cells
† As in Table 1. § As in Table 1.

A summary of the results of 105 individual experiments for splenic trapping and 127 experiments for lymph node trapping is also presented in Table 1. Although the range of trapping varies from experiment to experiment the phenomenon is reproduced with remarkable consistency. These results in C3H mice with sheep erythrocytes, KLH, and *Salmonella* H antigen conform well to the findings of Zatz and Lance (1971a) in CBA mice.

DEPLETION OF 'T' CELLS

Antilymphocyte serum is known to deplete discriminately thymus-derived recirculating small lymphocytes (Lance, 1970; Taub, 1970). Histological study of ALS-treated animals

TABLE 4

EFFECT OF CYCLOPHOSPHAMIDE ON ANTIGEN-INDUCED TRAPPING IN THE SPLEEN AND LOCAL NODE

	Experiment number 75: 200 mg/kg			Experiment number 78 200 mg/kg		Experiment number 87 300 mg/kg		Experiment number 105 200 mg/kg × 3 days	
Mean percentage localization of ^{51}Cr-labelled cells†	Control	SRBC-SC	SRBC-IV	Control	SRBC-IV	Control	SRBC-IV	Control	SRBC-IV
Lymph node: L	3·3 ± 0·4	4·8 ± 0·6							
R	3·2 ± 0·2	2·6 ± 0·4	12·8 ± 1·2	16·7 ± 2·1	14·1 ± 1·5	16·1 ± 1·5	9·7 ± ·21	21·2 ± 0·5	17·0 ± 1·0
M	9·2 ± 0·8	9·6 ± 1·0							
Spleen	11·3 ± 0·6	12·1 ± 0·7	19·0 ± 0·3	9·6 ± 0·2	13·2 ± 1·2	9·2 ± 0·6	15·2 ± 1·6	10·2 ± 0·5	15·1 ± 1·2
Liver	14·2 ± 0·7	14·2 ± 0·6	10·6 ± 0·5	11·9 ± 0·6	10·2 ± 0·4	15·5 ± 0·2	9·5 ± 1·5	19·3 ± 1·3	11·2 ± 0·7
Percentage trapping§		45% (L node)	68% (spleen)		37% (spleen)		65% (spleen)		48% (spleen)

† As in Table 1. § As in Table 1.

All groups were treated with cyclophosphosphamide at the dosage indicated and in addition some groups received antigen, prior to syngeneic ^{51}Cr-labelled lymph node cells.

L = Left sided lymph nodes. R = right sided lymph nodes. M = mesenteric lymph nodes.

TABLE 5

EFFECT OF HYDROCORTISONE ON ANTIGEN-INDUCED LYMPHOCYTE TRAPPING*

Mean percentage localization of ^{51}Cr-labelled cells†	Experiment number 119		Experiment number 148		Experiment number 192	
	No antigen	SRBC-IV	No antigen	SRBC-IV	No antigen	SRBC-IV
Lymph node	13·3±0·5	13·9±0·8	11·0±0·7	8·3±0·3	12·4%0·3	11·8±0·4
Spleen	17·8±0·6	22·8±0·6	17·7±1·0	21·6±1·3	14·6±0·7	19·3±0·9
Liver	17·2±0·4	11·4±0·8	14·6±0·4	11·0±0·4	16·4±1·1	15·2±0·5
Percentage trapping§ (spleen)		28%		22%		32%

* All groups received 2·5 mg hydrocortisone acetate intraperitoneally 48 hours before the administration of antigen, followed 1 hour later by syngeneic ^{51}Cr-labelled lymph node cells.
† As in Table 1. § As in Table 1.

showed marked depletion of lymphoid cells from the thymus-dependent areas of the lymph node and spleen. Labelled syngeneic lymphocytes injected into ALS-treated recipients show a different pattern of localization from that observed in untreated animals (Table 2). The decrease in lymph node homing and increase in localization in the liver are characteristic changes as has been previously described (Taub and Lance, 1969). Nonetheless the capacity to trap was unaltered (Table 2) with respect to two different antigens.

Animals severely depleted of 'T' cells by adult thymectomy, lethal irradiation and bone marrow reconstitution were also competent with respect to trapping (Table 3).

TABLE 6

RADIO RESISTANCE OF ANTIGEN-INDUCED LYMPHOCYTE TRAPPING*

Mean percentage localization of ^{51}Cr-labelled cells†	400 rad	700 rad	1000 rad	1250 rad	1500 rad
			Intravenous antigen		
No antigen	6·1±0·3	5·9±0·8	5·6±0·3	9·4±1·1	7·0±0·7
5×10^8 SRBC	9·0±0·5	9·0±0·3	9·6±1·2	13·3±0·4	11·4±1·3
Percentage trapping in spleen‡	47%	53%	71%	41%	62%
			Subcutaneous antigen		
Non-antigen stimulated nodes	4·5±0·8	4·7±0·8	3·5±0·3	2·7±0·4	
Antigen stimulated nodes	6·3±0·9	6·1±0·3	5·2±0·1	4·0±0·5	
Percentage trapping in lymph node§	40%	30%	48%	48%	

*Irradiation was administered 24 hours prior to antigen followed after 1 hour by syngeneic ^{51}Cr-labelled lymph node cells. Experimentation on the day of irradiation or a delay of up to 72 hours did not alter these results. Trapping after irradiation was demonstrable equally with KLH and *Sal.* H as the stimulating antigens.
† As in Table 1; § As in Table 1.

TABLE 7

RADIO RESISTANCE OF ANTIGEN-INDUCED LYMPHOCYTE TRAPPING IN 'B' MICE GIVEN AN ADDITIONAL 1000 RAD WHOLE BODY IRRADIATION

Mean percentage localization†	1000 rad, 'B' no antigen	1000 rad, 'B' 5×10^8 SRBC
Spleen	*12·5 ± 1·0	20·2 ± 1·3
Percentage trapping§		61% (spleen)

* Mean of two experiments.
† As in Table 1. § As in Table 1.
'B' mice which had received 900 rad previously were given an additional 1000 rad 24 hours prior to antigen challenge which was followed by one hour with syngeneic ^{51}Cr-labelled lymph node cells.

DEPLETION OF 'B' CELLS

Selective 'B' cell depletion with cyclophosphamide at a dosage of 200–300 mg/kg did not interfere with capacity to trap lymphocytes either in the spleen or lymph nodes (Table 4). Doubling of the cyclophosphamide dosage, e.g. 600 mg/kg administered over a 3-day period prior to antigen injection was equally ineffective in blocking trapping. Histological examination of cyclophosphamide treated animals showed depletion of lymphocytes in the lymph node and spleen comparable to those described by Turk and Poulter (1972).

TABLE 8

EFFECT OF PRESENSITIZATION ON TRAPPING BY BOVINE GAMMA GLOBULIN

Mean percentage localization of labelled cells:†	Control, no treatment	0·5 mg BGG intravenously	Freund's complete adjuvant subcutaneously	Freund's complete adjuvant/BGG challenge (subcutaneously) with 30 μg BGG 10 days later
Spleen	*11·2 ± 0·5	11·7 ± 0·5	10·4 ± 0·2	15·4 ± 0·3
Percentage trapping§		0	0	38

* Mean of ten experiments. † As in Table 1. § As in Table 1.
Four groups of three animals were either not treated; given 0·5 mg bovine gamma globulin (BGG) intravenously; given 0·25 cc Freund's complete adjuvant emulsified with saline subcutaneously 10 days earlier. Animals given Freund's adjuvant with bovine gamma globulin (BGG) subcutaneously 10 days earlier were challenged with 30 μg of bovine gamma globulin.

INDISCRIMINATE LYMPHOCYTE DESTRUCTION

Hydrocortisone acetate in large doses was totally without effect on trapping with a variety of antigens (Table 5). Whole body irradiation ranging from 400 to 1500 rad were given to animals from 6 to 72 hours prior to antigen injection. The results of twenty-two experiments of this kind are summarized in Table 6. Within the dose, range and timing used for these experiments no effect on the magnitude of trapping in spleen or draining

TABLE 9

EFFECT OF NON-ANTIGENIC PARTICLES ON LYMPHOCYTE TRAPPING

Mean percentage localization of ^{51}Cr-labelled cells†	Control	Latex particles intravenous	Control	Silica intravenous	Carrageenan* 25 mg subcutaneously	Control	Carbon intravenous
Lymph node	20·9±1·2	20·1±0·5	17·8±1·0	14·6±0·6 17·6±0·5	L 5·5±0·5 R 3·3±0·2 M 12·4±0·5	15·9±0·3	13·8±0·2
Spleen	14·1±0·9	19·5±0·8	11·5±0·8	17·6±0·5	13·8±0·4	13·3±0·8	24·5±0·7
Liver	15·5±0·6	13·4±0·8	18·5±0·6	5·4±0·2	17·1±0·9	19·4±0·5	10·2±0·2
Percentage trapping§		38% (spleen)		52% (spleen)	66% (lymph node)		87% (spleen)

* Carrageenan can be administered subcutaneously or intraperitoneally but is extremely toxic when given intraperitoneally even at low dosages. Non-immunogenic particulate materials were given intravenously 1 hour before syngeneic ^{51}Cr-labelled cells. In this experiment carrageenan was given 4 days prior to experimentation.

† As in Table 1. § As in Table 1.

TABLE 10

SUMMARY AND CLASSIFICATION OF MATERIALS TESTED FOR THEIR ABILITY TO INDUCE LYMPHOYCTE TRAPPING

Group I	Group II	Group III	Group IV	Group V
Sheep red blood cells	Latex	Vitamin A palmitate	Bovine gamma globulin	Syngeneic
Keyhole limpet haemocyanin	Silica	Vitamin A alcohol	Bovine serum albumin	(a) sera
Salmonella H	Carbon	Freund's complete adjuvant	Tetanus toxoid	(b) red cells
Pneumococcal polysaccharide	Carrageenan	Freund's incomplete adjuvant		(c) spleen cells
Tumour cells		Pertussis		Allogeneic sera
Tumour extracts		Endotoxin		Mineral oil
Xenogeneic sera		*Corynebacterium parvum*		
Heat-aggregated bovine gamma globulin				
Allografts and xenografts of skin				

Groups I–III, cause trapping in virgin animals. Group IV, cause trapping only in presensitized animals. Group V, do not cause trapping.

lymph nodes was apparent. In fact, the mean percentage trapping in the irradiated groups of animals was 50 per cent.

The experiments summarized in Table 6 were all performed using sheep erythrocytes as antigen but this same observation was valid when animals were challenged with KLH or *Salmonella* H antigen.

A striking finding was that 'B' mice subjected to an additional 1000 rad of whole body irradiation just prior to experimentation remained fully competent with respect to trapping (Table 7).

Categorization of agents which elicit trapping

Earlier work which is here corroborated demonstrated the ease and reproducibility with which large molecular weight or particulate and generally good immunogens could elicit trapping. We therefore tested a group of poor and/or soluble immunogens such as bovine gamma globulin, bovine serum albumin and tetanus toxoid to determine whether trapping would occur. These agents failed to cause trapping in virgin animals. However animals which had been presensitized to these antigens by immunization in conjunction with Freund's complete adjuvant did demonstrate trapping upon subsequent rechallenge (Table 8). The specificity of trapping under these circumstances is shown by the fact that preimmunization with Freund's complete adjuvant alone 10 days prior to the injection of labelled cells does not of itself cause trapping and, moreover, animals preimmunized with Freund's complete adjuvant and an unrelated antigen do not show trapping when challenged with bovine gamma globulin. Furthermore, preimmunization with Freund's complete adjuvant/bovine gamma globulin will not enable an antigen such as bovine serum albumin to initiate trapping.

Because of the observations that particulate antigens were effective we tested the ability of a variety of particulate but presumably non-immunogenic materials in this system. Studies with latex particles, silica, carbon and carrageenan are summarized in Table 9. All such materials were potent initiators of this response.

Table 10 summarizes our experience on the nature of substances which can or cannot initiate trapping in virgin animals.

DISCUSSION

Under normal circumstances there is a balanced flux of lymphocytes across the afferent and efferent terminals of the central lymphoid organs. The various compartments are in kinetic equilibrium. This normal state of affairs is altered considerably after the administration of immunogens. The early rapid rise in lymphocyte content of stimulated lymphoid organs appears to be a clear consequence of this change in traffic dynamics rather than the result of cell replication (Taub and Gershon, 1972). The anatomic mechanism which mediated this function is unknown. The lymphoid plugs which have been described by de Sousa and Parrott (1969) imply an obstruction of flow through lymphoid sinusoids. Vascular changes are known to occur during immunization and marked enlargement of the capillary and post capillary venules have been recently described by Herman, Yamamoto and Mellins (1972) in the immunized lymph node.

Earlier work by Knisely (1934, 1936) may provide a model from which analogy can be drawn. He studied the blood flow through the isolated rat spleen by transillumination and

described a sphincteric mechanism in the red pulp which operated in the following sequence. The initial phase was a closure of the efferent sphincter following which the sinusoids became packed and engorged with red cells after which the presinusoidal sphincter also closed. These enclosed sinusoidal 'packets', which resemble lymphoid plugs, could remain closed for as long as 10 hours after which the post sinusoidal venule sphincter opened and the sinusoid emptied. This was followed by opening of the presinusoidal arteriolar sphincter and the resumption of normal recirculation. If some similar vascular apparatus exists which regulates the flow through lymphatic sinusoids, its triggering mechanism remains to be explained.

At the beginning of our studies it seemed a reasonable assumption that an immunologically competent cell, e.g. a lymphocyte, would be central in initiating trapping. In view of the marked resistance of trapping to procedures which either selectively or indiscriminately destroy lymphocyte populations, this possibility is no longer tenable.

A variety of circumstantial evidence is consistent with the possibility that the macrophage plays a central role in initiating trapping. Macrophages are known to be more radio-resistant *in vivo* than lymphocytes (Perkins, Netteschains and Morita, 1966; Fleming, Fleming and Nothdurft, 1970). Although irradiation and cyclophosphamide may inhibit the ability of macrophages to metabolize ingested material they do not significantly affect phagocytosis (Benacerraf, Kiu, Rosenberg, Sebesetyen and Zweifach, 1959; Sharbaugh and Grogan, 1969; Fleming *et al.*, 1970; Gallily and Feldman, 1967). Both low dose irradiation and some antilymphocytic sera are known to stimulate phagocytosis (Grogan, 1969; Argyris and Plotkin, 1970; Kinneart, Mahieu and Penneman, 1972). The effect of corticosteroids of the cortisol type on the reticulo-endothelial system has not been systematically investigated. Phagocytosis in rabbits does not appear to be affected although lysosomal membranes (Haugen, Bassoe and Flood, 1969) may be rendered more stable. These observations suggest that macrophage function might not be seriously deranged by the immunosuppressive manoeuvres to which the trap is known to be resistant.

The fact that particulate and/or high molecular weight substances are highly effective in causing trapping regardless of their immunogenicity while soluble proteins are inefficient, is compatible with a macrophage-dependent phenomenon. The greater efficiency with which trapping operates in presensitized animals requires some explanation. Antigens such as BGG and BSA which do not trap in virgin animals are perfectly capable of inducing trapping in presensitized hosts. Trapping after the injection of sheep erythrocytes is a dose-dependent phenomenon and in preimmunized animals can be achieved with far lower doses (Zatz and Lance, 1971a; Frost, unpublished data). In as much as both these changes are immunologically specific they require that macrophages become immunologically armed, e.g. cytophilic antibody, to be able to fulfil this function. We have demonstrated recently that passively administered mouse anti-BGG serum is as effective as active presensitization in allowing animals to trap after BGG (Frost and Lance, unpublished data).

Tolerance to transplantation antigens (Zatz and Lance, 1971b) and to BGG (Frost and Lance, 1973) eliminates in an immunologically specific way the capacity to trap. These observations pose no problems with respect to BGG in as much as the absence of an immune response would preclude arming macrophages with the postulated necessary cytophilic antibody. The failure of tolerant animals to show trapping after the application of skin graft may be slightly more complex. Trapping during normal allograft rejection is a late event arising in the second week after transplantation at a time when lymphoid infiltration

and graft destruction are already well advanced (Zatz and Lance, 1971a). If we assume these steps are necessary to liberate the antigenic debris which stimulates macrophages in the draining lymph node then the central role attributed to the macrophage remains unblemished.

Further evidence comes from studies in which the capacity to trap to BGG was adoptively transferred to virgin animals by various cell populations. In these studies peritoneal exudate cells and the adherent component of spleen cells from animals presensitized to BGG were the most effective populations in conferring capacity to trap on virgin animals (Frost and Lance, 1973).

Although the evidence is consistent with the possibility that macrophages are primarily responsible for springing the lymphocyte trap the data is circumstantial and awaits direct proof. In the meantime alternative possibilities such as the mediation of trapping by vascular endothelial cells although unlikely (van Furth, Cohen, Hirsch, Humphrey, Spector and Langevoort, 1972) cannot be absolutely excluded.

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