Thoracic Duct Lymphocytes in Experimental Lepromatous Leprosy in Mice

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Progress in leprosy research was very restricted until recently, for it was, and still is, impossible to grow the causative organism *Mycobacterium leprae in vitro*. The work of Shepard ([5]), however, opened up new possibilities, for he demonstrated that *M. leprae* can be grown in the footpads of mice. Rees and Weddell ([2,3]) and colleagues have further contributed to experimental leprosy in mice for they have shown that in mice, as in men, the disease depends upon the immunologic capacity of the individual. Mice act as experimental models for the production of different forms of leprosy, which correspond to those in patients in Ridley and Jopling's classification scale ([4]). In particular, they have shown that the lesions in mice, indistinguishable from those of lepromatous leprosy in man, can be evoked by reducing their immunologic capacity by thymectomy and 900r total-body irradiation prior to infection. They have further shown that it is possible to produce an immunologic upgrading or a reversal reaction in heavily infected mice if a total syngeneic lymphoid tissue replacement, originating from the spleen and other sources of lymphoid tissue, is given to the animal after infection with *M. leprae*. However, this lymphoid replacement tissue contained, in addition to macrophages and other cells, a mixed population of at least two types of lymphocyte, viz., thymic-dependent and bone marrow-derived cells. It was thus possible that the reactions observed were due not solely to long lasting thymic-dependent lymphocytes but to the spectrum of cells which was given. The present study was therefore designed to determine the role of syngeneic thoracic duct lymphocytes, the majority of which are thymic-dependent.

MATERIAL AND METHODS

CBA strain mice were thymectomized at six weeks of age and then subjected to 900r total-body irradiation followed immediately by an intravenous injection of 0.3 ml. of syngeneic bone marrow. After allowing three weeks for the animals to recover they were inoculated into both hind footpads with 10^5 to 10^7 *M. leprae* from patients with lepromatous leprosy. Six to eight months later, animals with swollen footpads were selected as lepromatous recipients. Subsequent histologic observations showed that this method of selection had been successful, for all the mice had lesions which were indistinguishable from those in patients with lepromatous leprosy.

The lymphocytes were obtained by cannulating the thoracic ducts of normal CBA mice donors by a modification of the technique of Boak and Woodruff ([1]). The harvested lymphocytes were labelled *in vitro* with 3H uridine (15μc/ml) and a final suspension of the cells was adjusted to a concentration of $16 - 20 \times 10^7$ ml.; 0.3 ml of this suspension was injected into the tail veins of lepromatous mice. Normal CBA mice served as controls.

In addition, long-lived populations of small lymphocytes were labelled *in vivo* by injecting a series of CBA mice with 3H thymidine (0.75 μc/gm. body weight) daily for 17 days. Three weeks later their thoracic ducts were cannulated; the lymphocytes were harvested, their concentration was adjusted $16 - 20 \times 10^7$ ml., and the suspension was injected into lepromatous mice. A lepromatous mouse which had not received lymphocytes served as a control (Table I).

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TABLE 1. *Details of experiment*

Serial no.	Number of animal	Dose and source of *M. leprae*	Route of infection	Duration of infection (months)	Isotope used for labelling lymphocytes	Survival periods of animals after lymphocytes injected (hours)	Remarks
Normal animals:							
1	NCBA/1	(normal) nil	nil	nil	TUD	2	Control animal
2	NCBA/2	" "	"	"	TUD	3	" "
3	NCBA/3	" "	"	"	TUD	4	" "
4	NCBA/4	" "	"	"	TUD	7	" "
T/900r Lepromatous animals:							
5	CBA 774/1231	10^5, mouse passage	B.F.P.	11	No lymphocytes injected	—	ARG control
6	CBA 1219A0	10^5, mouse passage	B.F.P.	10½	TUD	4	—
7	CBA 856/1231	10^5, mouse passage	B.F.P.	11	TUD	5	Thymus graft 2½ months
8	CBA 809/1231	10^7, mouse passage	I.V.	9	TTD	6	—
9	CBA 1184/1433	10^6, Maltese patient	B.F.P.	15	TUD	8	—
10	CBA 898/1231	10^7, mouse passage	I.V.	9	TUD	9	—
11	CBA 1231/884	10^5, mouse passage	B.F.P.	10½	TUD	9	—
12	CBA 857/1231	10^5, mouse passage	B.F.P.	11	TUD	10	Thymus graft 2½ months
13	CBA 1219/B	10^5, mouse passage	B.F.P.	10½	TUD	12	—
14	CBA 897/1231	10^7, mouse passage	I.V.	9	TTD	12	—
15	CBA 1/1219A	10^5, mouse passage	B.F.P.	10½	TUD	13	—
16	CBA 1219/A	10^5, mouse passage	B.F.P.	9½	TUD	16	—
17	CBA 859/1231	10^5, mouse passage	B.F.P.	11	TUD	16	Thymus graft 2½ months
18	CBA 2/1219A	10^5, mouse passage	B.F.P.	10½	TUD	18	—
19	CBA 1219C	10^5, mouse passage	B.F.P.	10½	TUD	24	—
20	CBA 899/1231	10^7, mouse passage	I.V.	9	TUD	24	—
21	CBA 1016/1238	3×10^9, Malaysian patient	I.V.	8	TTD	24	—
22	CBA 1219/1V	10^5, mouse passage	B.F.P.	12½	TTD	24	—
23	CBA 1219/2V	10^5, mouse passage	B.F.P.	12½	TTD	36	—
24	CBA 1219/3V	10^5, mouse passage	B.F.P.	12½	TTD	48	—
25	CBA 1219/4V	10^5, mouse passage	B.F.P.	12½	TTD	56	—

B.F.P. = Both Footpads; I.V. = Intravenous; TUD = 3H Uridine; TTD = 3H Thymidine.

The treated animals were killed at intervals ranging from two to 56 hours. Autoradiographs of various tissues were made and then examined under various combinations of incident and transmitted light, using a Leitz Ortholux microscope fitted with Ultrapak objectives. By this means it proved possible to trace the whereabouts in the infected mice of the labelled lymphocytes, and rough estimates were made as to their concentrations. To complement the histologic observations a proportion of the tissues examined were also processed for liquid scintillation counting.

OBSERVATIONS

Table 1 shows the details of the experiments and the immunologic status of the recipient mice and Table 2 the experiments in which tritiated uridine was used as a marker. Labelled cells were observed in the smears made from lymphocyte suspensions before injection. Blood smears and sections of spleen from recipient animals also displayed the presence of labelled lymphocytes (Figs. 1, 2 and 3). However, none of the labelled cells was seen in the sciatic nerve, footpad or nose, all of which were sites of lesions and all of which contained an excess of unlabelled lymphocytes (Fig. 4).

In Table 3 are shown details of the experiments in which the isotope tritiated thymidine was used. The results in these experiments were also negative except that in one animal, which survived for 56 hours, there was a solitary labelled lymphocyte in the perineurium of the sciatic nerve (Fig. 5).

Table 4 details two experiments in which, due to a technical fault, the labelled lymphocyte suspension injected contained, in addition, labelled cell debris. Table 5 gives details of the controls. Labelled cells are conspicuously absent in all tissues in these animals. The fact that labelled lymphocytes were present in the peripheral blood and spleen of the recipient animals makes it certain that the experiments had succeeded from a technical view point. The

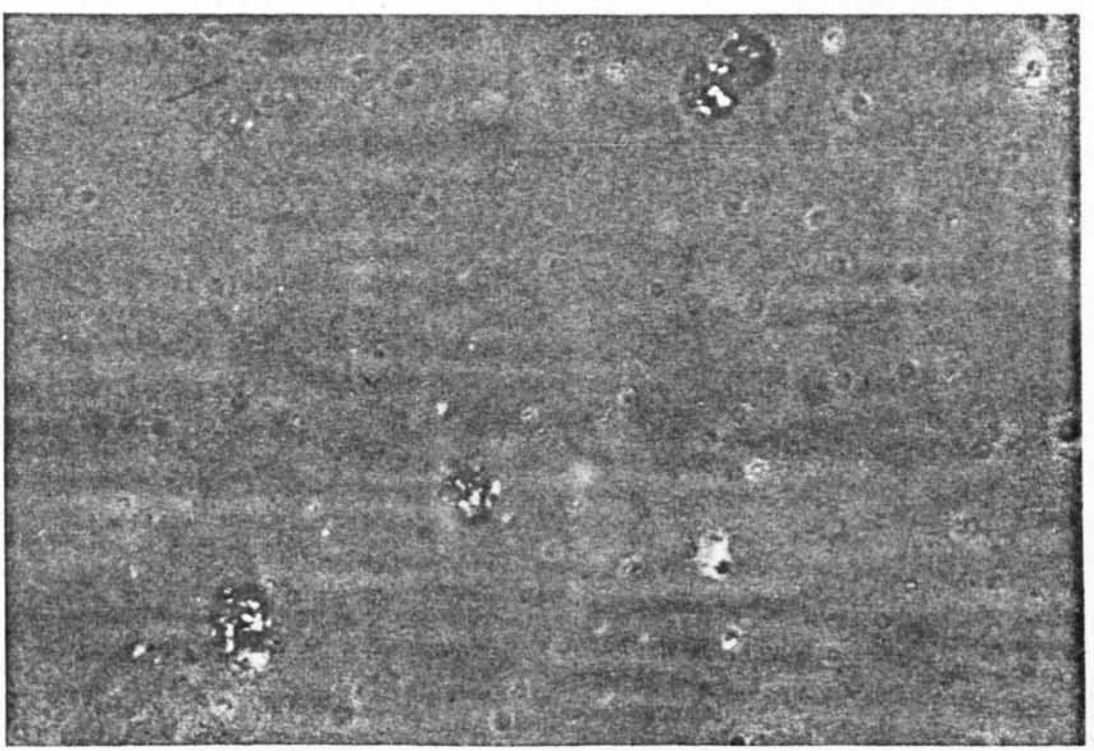

FIG. 1. Autoradiograph. Uridine-labelled lymphocytes in smear from suspension, donor animal.

TABLE 2. *3H uridine labelled lymphocytes (thoracic duct), lepromatous mice*

Mouse no.	Survival time hours	Smear injn. cells	Blood mear	Sciatic nerve	Foot pad	Spleen	Thymus	Status of infection H.&F.F. staining		Remarks
								Footpad	Sci. nerve	
CBA 1219A0	4	+	++	○	○	+	not present	not exam.	not exam.	—
CBA 856/1231	5	+	++	⊕	○	not taken	○	Lymphos +	Lymphos +	Labelled cell blast type. Thymus grafted beneath kidney capsule 9 months after infection 2½ months before killing.
CBA 898/1231	8	+	+	+	○	+	not present	not exam.	not exam.	Lymphocytes labelled in wall blood vessel in perineurium.
CBA 884/1231	9	+	+	○	○	not taken	not present	not exam.	Lymphos +	—
CBA 857/1231	10	+	+	○	○	not taken	○	not exam.	Lymphos +	Thymus grafted beneath kidney capsule 9 months after infection 2½ months before killing.
CBA 1219B	12	+	+	○	○	+	not present	not exam.	not exam.	—
CBA 1/1219A	13	+	+	○	○	not taken	not present	not exam.	not exam.	—
CBA 859/1231	16	+	?	○	○	not taken	⊕	Lymphos +	Lymphos +	Thymus graft as in 5 & 10 hours. Labelled cells two blast type.
CBA 2/1219A	18	+	+	○	○	not taken	not present	not exam.	Lymphos +	—
CBA 1219C1	24	+	?	○	○	+	not present	Lymphos +	Lymphos +	Blast cells labelled in spleen.
CBA 899/1231	24	+	?	○	○	+	not present	not exam	not exam.	—

+ = labelled lymphocytes present. ○ = no labelled cells.
⊕ = labelled cells not lymphocytes. ? = difficult to find or doubtful.

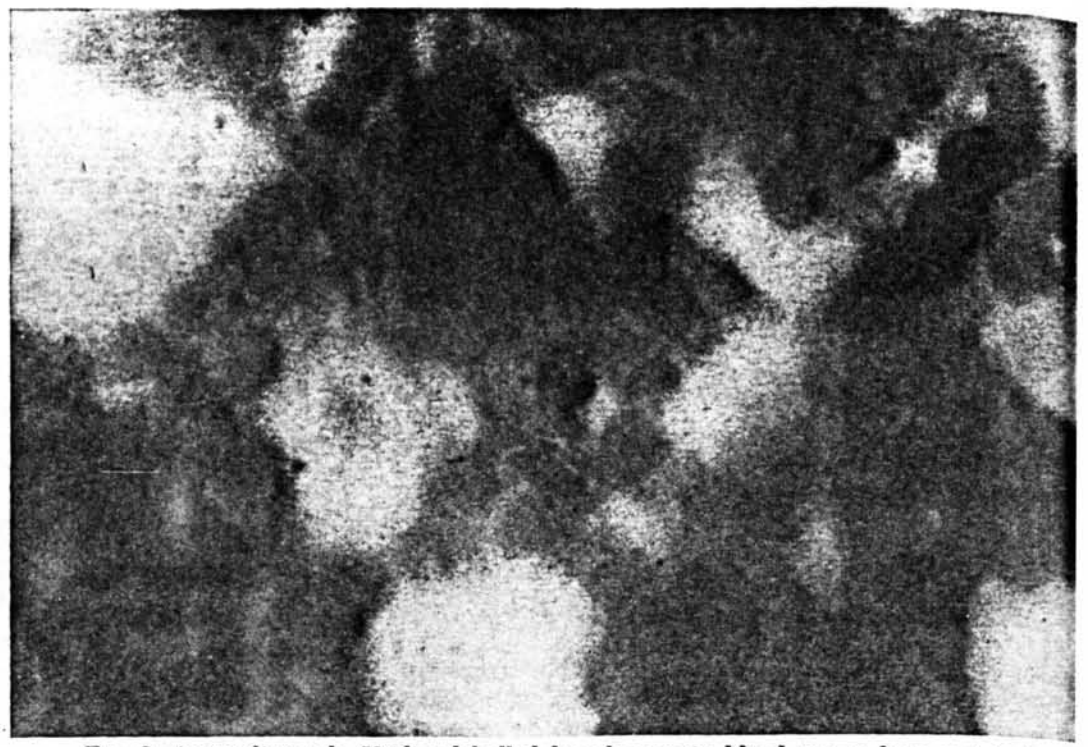

FIG. 2. Autoradiograph. Uridine-labelled lymphocyte in blood smear from recipient animal.

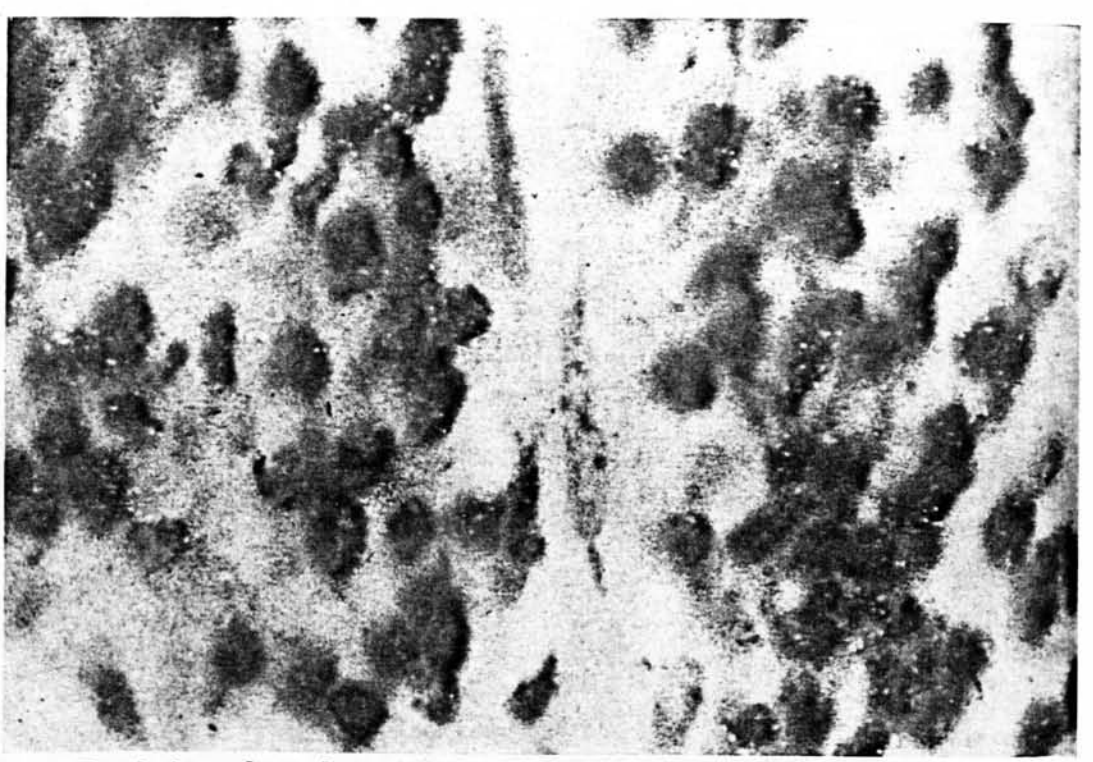

FIG. 3. Autoradiograph. Uridine-labelled lymphocytes in section of spleen, recipient animal.

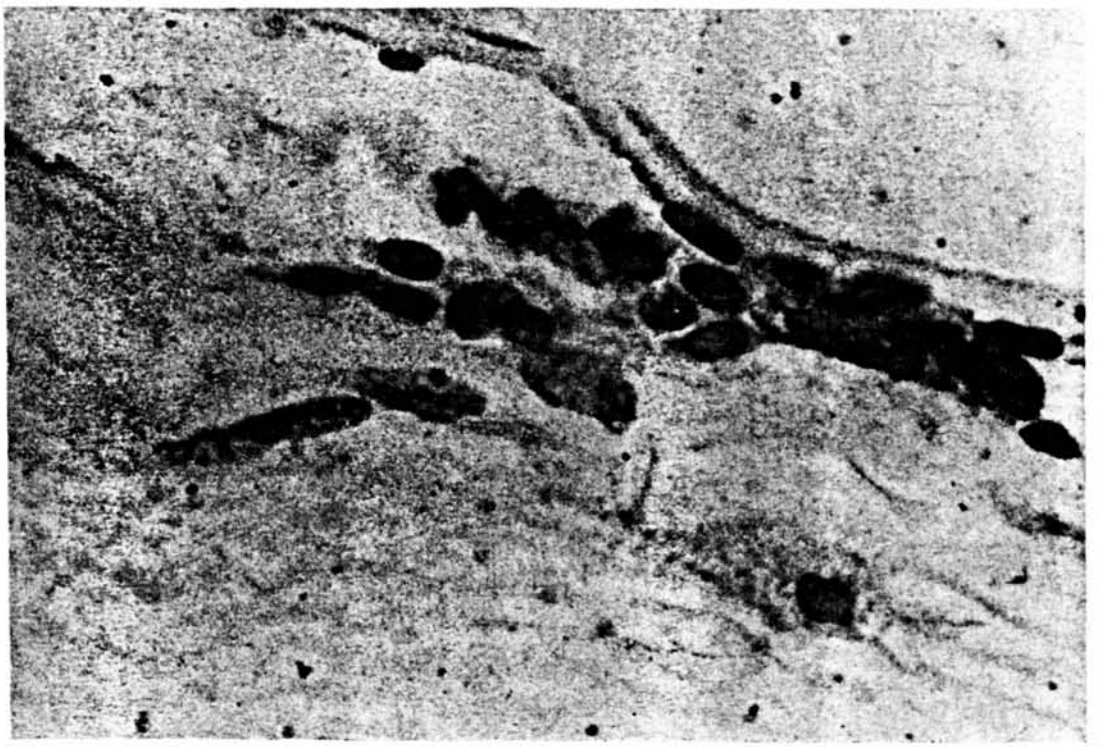

FIG. 4. Unlabelled lymphocytes in perineurium of sciatic nerve, recipient animal.

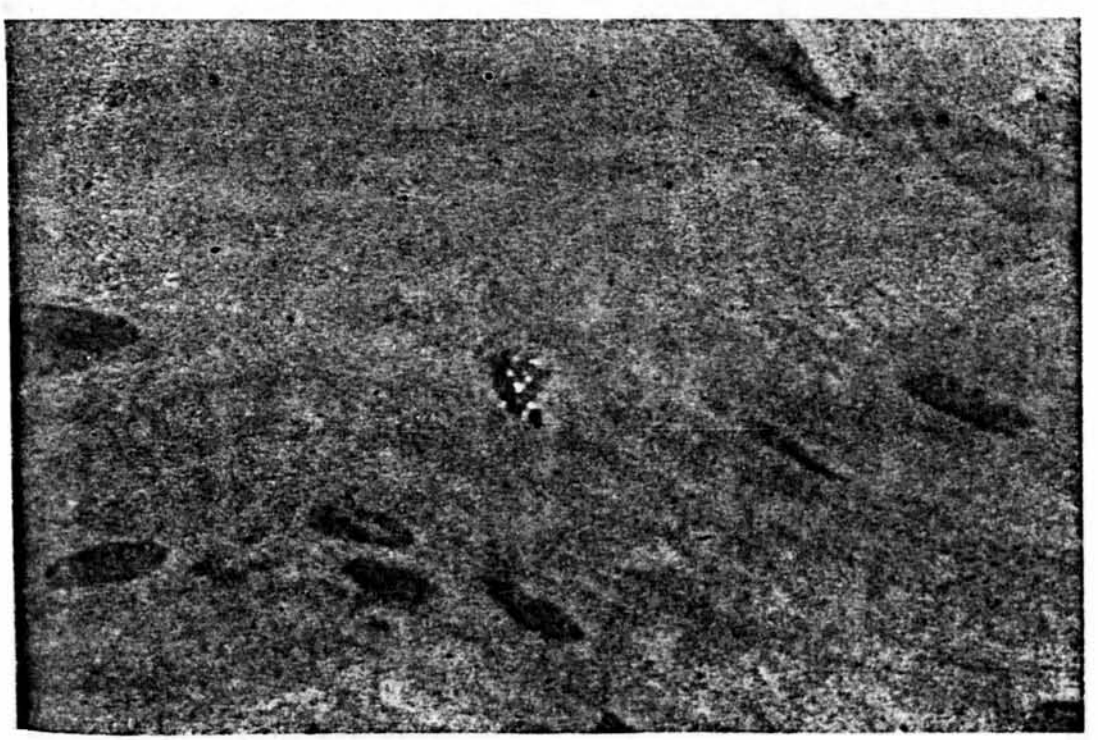

FIG. 5. Autoradiograph. Solitary thymidine-labelled lymphocyte in perineurium of sciatic nerve, recipient animal, 56 hours after injection of labelled cells.

TABLE 3. *3H thymidine labelled lymphocytes (thoracic duct), lepromatous mice*

Mouse no.	Survival time hours	Smear injn. cells	Blood smear	Sciatic nerve	Foot pad	Spleen	Status of infections H.&F.F. staining		Remarks
							Footpad	Sci. nerve	
CBA 809/1231	6	+	+	○	○	technical failure	not exam.	not exam.	—
CBA 897/1231	12	+	?	○	○	+	not exam.	not exam.	—
CBA 1016/1238	24	+	?	○	○	+	not exam.	not exam.	—
CBA 1219/IV	24	+	?	○	○	not exam.	+	+	One labelled in perineurium near blood vessel. Many unlabelled in endoneurium.
CBA 1219/2V	36	+	?	○	○	not exam.	+	+	—
CBA 1219/3V	48	+	?	○	○	not exam.	+	+	—
CBA 1219/4V	56	+	?	+ (one)	○	+	+	+	—

+ = labelled lymphocytes present.
○ = no labelled cells.
? = difficult to find or doubtful.

TABLE 4. *3H uridine labelled lymphocytes and excess label and medium injected in the peritoneal cavity of lepromatous mice*

Mouse no.	Survival time hours	Smear injn. cell	Blood smear	Sciatic nerve	Foot pad	Spleen	Lymph node	Ilium	Heart	Status of infection H.&F.F. staining		Remarks
										Foot-pad	Sci. nerve	
CBA 1184/1433	8	+ (a few)	○	⊕	⊕	+ diffuse	not exam.	not exam.	+ diffuse	not exam.	not exam.	Really a technical failure
CBA 1219A	16	++	++	○	○	++++	++++	++	++⊕	lepro-matous	lepro-matous	Heart contained labelled fibroblasts as well as labelled lymphocytes.

\+ = Labelled lymphocytes present.
○ = no labelled cells.
⊕ = labelled cells not lymphocytes.

TABLE 5. *3H uridine labelled lymphocytes (thoracic duct), controls—normal animals*

Mouse no.	Survival time hours	Blood	Sciatic nerve	Footpad
NCBA /1	2	not exam.	○	○
NCBA /2	3	not exam.	○	○
NCBA /3	4	not exam.	○	○
NCBA /4	7	OL	○	○
Lepromatous mouse (no labelled cells injected)				
CBA 774/1231	—	OL	L	L

○ = no labelled cells.
L = lymphocytes not labelled.

liquid scintillation counts confirmed the observations by light microscopy.

DISCUSSION

The result of these experiments was negative. That is to say labelled syngeneic mouse thoracic duct lymphocytes do not gather in leprosy lesions in recipient mice within 56 hours of their injection into the blood stream.

The reasons for this are not yet clear. It may be that the period tested was too short for thoracic duct lymphocytes to become concentrated in the lesions. This question of time is currently under investigation, but even if the results of long term experiments also prove negative there are clearly further experiments which can be designed around the techniques described above. By these means it should be possible, in time, to determine the source or sources of the lymphocytes which take part in the cell-mediated immune response which occurs in mice infected with leprosy.

SUMMARY

For these studies a leprosy infection was established by the inoculation of 10^5 – 10^7 *M. leprae* into the hind footpads of female CBA strain mice that had been thymectomized at six weeks of age, followed three weeks later by total-body irradiation (900r) followed immediately by an intravenous injection of syngeneic bone marrow. Six to eight months after inoculation animals with swollen footpads were selected, since histologic examination showed pictures indistinguishable from those in patients with lepromatous-type leprosy. Mice with these established infections were inoculated intravenously with either *in vitro* or *in vivo* labelled lymphocytes obtained by cannulation of the thoracic ducts of normal CBA mice.

Thoracic duct lymphocytes were obtained from normal CBA mice by a modification of the technique of Boak and Woodruff[1]. The harvested lymphocytes were labelled with 3H uridine (15 μ/ml.) and inoculated intravenously at a concentration of 5–7 x 10^7/mouse using both normal and leprosy animals. Long-lived populations of small lymphocytes were also labelled *in vivo* by the injection of normal CBA mice with 3H thymidine (7.5 μ/gm. body weight) daily for 17 days, and 5–7 x 10^7 such cells were inoculated into leprosy mice and their distribution was compared with that in similar mice uninoculated with lymphocytes.

The lymphocyte-inoculated animals were killed at intervals ranging from two to 56 hours. The whereabouts of the labelled lymphocytes were followed by autoradiography. The results of these studies and their significance are discussed.

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