

THE CARRIAGE OF IMMUNOLOGICAL MEMORY BY
SMALL LYMPHOCYTES IN THE RAT*

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Although it has been shown that antibody-forming cells arise during secondary responses from dividing precursors (1-3), the origin of these precursors is still a matter of debate. In general terms they may either originate from a line of dividing cells which is formed during primary immunization, or from long-lived cells, also formed during primary immunization, but which only begin to divide after secondary challenge. Attempts have been made to distinguish between these alternative explanations of "immunological memory" by giving a pulse of tritiated thymidine immediately before secondary challenge and determining whether or not the antibody-forming cells become labeled. Experiments of this kind led Nossal and Mäkelä (4) to conclude that the cells which formed antibody after challenge with *Salmonella adelaide* in rats arose from large lymphocytes which were already dividing in the animal before the antigen had been given. On the other hand, Cohen and Talmage (5) concluded that the cells which synthesized antibody in response to a secondary challenge with bovine gamma globulin in mice were not dividing in the animal before the challenge; they questioned the conclusions of Nossal and Mäkelä on the grounds that reutilization of label might have accounted for their findings. A similar criticism has been made by Mitchell et al. (6).

The most obvious candidate for the long-lived, nondividing carrier of immunological memory is the small lymphocyte (7-9). Evidence consistent with this possibility was obtained by showing that heavily irradiated rats responded in a secondary manner to tetanus toxoid if they had been injected with thoracic duct lymphocytes from primarily immunized donors; but a possible contribution by the large dividing lymphocytes in lymph was not excluded (7). The present paper records similar experiments in rats immunized with bacteriophage ϕ X 174 in which strong evidence has been obtained for the carriage of immunological memory by small lymphocytes.

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Methods

The general plan of the experiments was as follows. Thoracic duct lymphocytes were collected from rats 1½ to 15 months after a single immunizing dose of ϕ X 174. The cells were injected intravenously into syngeneic hosts whose ability to respond actively to antigen had been virtually abolished by prior X-irradiation. The hosts were then challenged with antigen to determine the character of the immune response which had been conferred by the transferred cells. Finally, the response conferred by fresh thoracic duct cells was compared with that given by inocula of small lymphocytes from which the large, dividing lymphocytes had been removed.

Primary Immunization.—A highly purified preparation of bacteriophage ϕ X 174 (ϕ X) was obtained from Dr. R. L. Sinsheimer. Male and female rats, aged 2 to 3 months, of a highly inbred hooded (HO) strain were given a single dose of 10^{11} particles of ϕ X distributed subcutaneously between the four foot-pads, both flanks, the nape of the neck, and submentally.

Cell Transfer.—

“Fresh” thoracic duct cells: At various times after primary immunization, the thoracic duct of the donors was cannulated under ether anesthesia by the method of Bollman et al. (10). The rats were maintained, unanesthetized, in restraining cages and lymph was collected in successive 12-hr samples into sterile flasks each of which contained 5 ml of Krebs-Ringer solution, 100 units of heparin, and 0.5 mg streptomycin. A continuous intravenous infusion of Krebs-Ringer solution with 2 units heparin/ml and 100 μ g streptomycin/ml was run into the femoral vein of each rat under gravity at approximately 2 ml/hr.

Each sample of lymph was centrifuged at 100 g for 10 min and the cells were resuspended in Krebs-Ringer solution containing 2 units heparin/ml for injection into the recipients. These inocula will be referred to as “fresh” thoracic duct cells.

“Incubated” thoracic duct cells: To determine whether the effects which followed the injection of “fresh” thoracic duct cells were due solely to the activity of small lymphocytes, a number of experiments were performed with inocula in which most of the large and medium lymphocytes, which normally make up about 5 to 10% of the cells in lymph during the first 36 hr following cannulation, had been destroyed. The technique employed for this purpose made use of the fact that during incubation *in vitro* at 37°C for 24 hr with constant shaking, the larger lymphocytes die more rapidly than small lymphocytes (11). Thoracic duct cells from 12-hr collections of lymph were washed once in Krebs-Ringer solution and suspended at a concentration of 5 to 10×10^7 cells/ml in medium 199 to which had been added 20% v/v phosphate-buffered saline, pH 7.3 (Dulbecco “A”) and 1% inactivated rat serum. The mixture was incubated in 25 ml corked conical flasks at 37°C in a water bath with constant shaking. After incubation the cells were separated by centrifugation and resuspended for injection in the manner described for “fresh” thoracic duct cells. The “incubated” thoracic duct cells used in the present experiments contained from 0.05 to 0.6% of cells which could be regarded on grounds of morphology or size as being other than small lymphocytes. These estimates were made for each inoculum from a count of 2000 cells in smears stained with Wright’s stain.

The classification of lymphocytes in smears on the basis of differences in size and morphology is an arbitrary procedure. A more objective demonstration that large and medium lymphocytes are selectively destroyed by incubation *in vitro* was obtained in the following way. The thoracic ducts of two normal rats were cannulated and an intravenous infusion of tritiated thymidine (Radiochemical Centre, Amersham, England; specific activity 2.47 c/mole) was run continuously into the femoral vein of each at the rate of 10 μ c/hr for 24 hr. Radioautographs were prepared with Ilford K5 dipping emulsion of cells from the second 12-hr collection of lymph from each rat and Fig. 1 and Table I show that almost all the large and medium lymphocytes were labeled. Radioautographs were again prepared after a sample from each of the two collections of lymphocytes had been subjected to the incubation procedure. Fig. 2

and Table I show that many cells died during incubation but that the reduction in the total number of labeled cells was proportionally much greater. Only 0.15 and 0.4% respectively of all the cells remaining in the two cultures were identified as large and medium lymphocytes; these were all labeled.

Recipients: Lymphocytes were injected into the tail vein of syngeneic recipients which had received 500 rads of whole-body X-irradiation 24 hr previously. Some recipients were given a single injection of cells; others received up to 3 injections from the same donor spaced at approximately 12-hr intervals. A challenging dose of $10^{10} \phi X$ was given intravenously with the last or the only injection of cells. In each case where a comparison was made between the effects of fresh and incubated thoracic duct cells, pairs of recipients received cells from the same donor and were challenged with antigen at the same time after X-irradiation.

TABLE I
Effect of Incubation in Vitro for 24 hr at 37°C on Survival of Large and Medium Lymphocytes from Rat Thoracic Duct Lymph

Experiment No.	Lymphocyte cultures	Per cent labeled cells in count on 2000 total cells*		L and M lymphocytes labeled*, ‡	Total cells in culture surviving incubation§
		L and M	S		
1	Before incubation	13.0	0.8	100	67
	After incubation	0.15	1.4	—	
2	Before incubation	5.4	0.6	99.2	58
	After incubation	0.4	0.4	—	

L, large, M, medium, and S, small lymphocyte.

* From radioautographs of smears exposed for 28 days. Cells labeled by giving continuous intravenous infusion of tritiated thymidine to lymphocyte donor (see text).

† From count on 500 L and M lymphocytes before incubation.

§ From hemocytometer counts on cultures before and after incubation.

The recipients were bled at intervals after antigenic challenge and the sera which accumulated from each experiment were assayed in batches by the phage neutralization method (12). The specificity of neutralization of antisera from ϕX -immunized rats was established by the lack of significant neutralization by such antisera of an immunologically unrelated bacteriophage T_2 . Sera were also titrated after treatment with 2-mercaptoethanol (2-ME).

RESULTS

Primary and Secondary Antibody Response to ϕX in Rats

Table II shows that a single dose of $10^{11} \phi X$ evoked an easily detectable primary antibody response in rats and that a subsequent challenge with $10^{10} \phi X$ produced a secondary response with a titer (serum k), 1 to 2 wk after challenge, about 100 to 1000-fold greater than the primary. The majority of antibody obtained one week after primary immunization was inactivated by 2-ME and

was presumably 19S antibody (13). 1 wk after secondary challenge, the k values of the sera were as high or higher after 2-ME treatment indicating that much or all of the antibody was 7S.

It was of some interest that drainage of lymphocytes for 5 days from a thoracic duct fistula did not significantly diminish the late primary response to $10^{11} \phi X$ administered on the day following closure of the fistula, although the response at 1 wk (mainly 19S antibody) did appear to be somewhat lower (Table II). Depletion of lymphocytes by this method virtually abolishes the primary

TABLE II
Primary and Secondary Responses to Bacteriophage ϕX 174 in Normal Rats and Primary Response in Rats After Whole-Body X-Irradiation or Chronic Drainage of Lymphocytes from a Thoracic Duct Fistula

Rat No.	Treatment	Serum antibody (k) (days after immunization)		
		7	14	21
402/1	$10^{11} \phi X$ s.c.	1.1	3.1	8.2
		0.55	1.9	25.0
		0.67	0.07	7.5
		1.5	0.71	1.5
402/1	$10^{11} \phi X$ s.c.; 10^{10} i.v. 26 days later	150	200	180
		610	630	310
407	5 days' drainage from thoracic duct, then	0.22	8.1	5.5
408	$10^{11} \phi X$ s.c.	0.13	—	11
403/1	$10^{10} \phi X$ 24 hr after 500 rads X-ray	0.025	0.02	0.005
		<0.002	<0.001	<0.001

response of rats to sheep erythrocytes and to tetanus toxoid (14) and their ability to respond to ϕX is no doubt a reflection of the excellent immunogenicity of this antigen. A similar difference between the response of lymphocyte-depleted rats to strong and weak antigens has also been observed in reactions to homografts of skin (15). There is at present no satisfactory explanation of the difference between strong and weak antigens in terms of the cellular mechanisms underlying the responses of animals to them. The problem has been discussed by Simonsen (16).

Response to ϕX of X-Irradiated Rats after Transfer of Lymphocytes from Immunized Donors

The thoracic ducts of rats were cannulated $1\frac{1}{2}$ to 15 months after primary immunization with $10^{11} \phi X$ at a time when the level of circulating antibody had

reached a plateau or was declining. In each experiment the lymphocytes were injected intravenously into X-irradiated syngeneic recipients which were then challenged with 10^{10} ϕ X by the same route. Table II shows that when no cells were transferred this challenging dose of antigen evoked a negligible antibody response.

Cell Transfer 2 Months after Immunization.—The response of 5 irradiated rats which were challenged with ϕ X after cell transfer is shown in Table III. The injection of lymphocytes from donors immunized 2 months previously enabled

TABLE III
Comparison of Antibody Responses in X-Irradiated Rats Given 10^{10} ϕ X and either "Fresh" or "Incubated" Thoracic Duct Cells from Donors Immunized 2 Months Previously

Rat No.	Donor lymphocytes		Serum antibody (k) (days after challenge)			Donor No.
	i.v. dose ($\times 10^6$)	Status*	7	14	21	
Recipients, ‡ 412/1	4.5	f	46	29	61	D1
	4.3	i	120	250	100	
	5	i	100	180	50	
	3	f	280	390	160	D3
	6	i	610	520	—	
			1700	290	320	
Donors, § D1			310	260	130	
D2						

* f, "fresh"; and i, "incubated" thoracic duct cells.

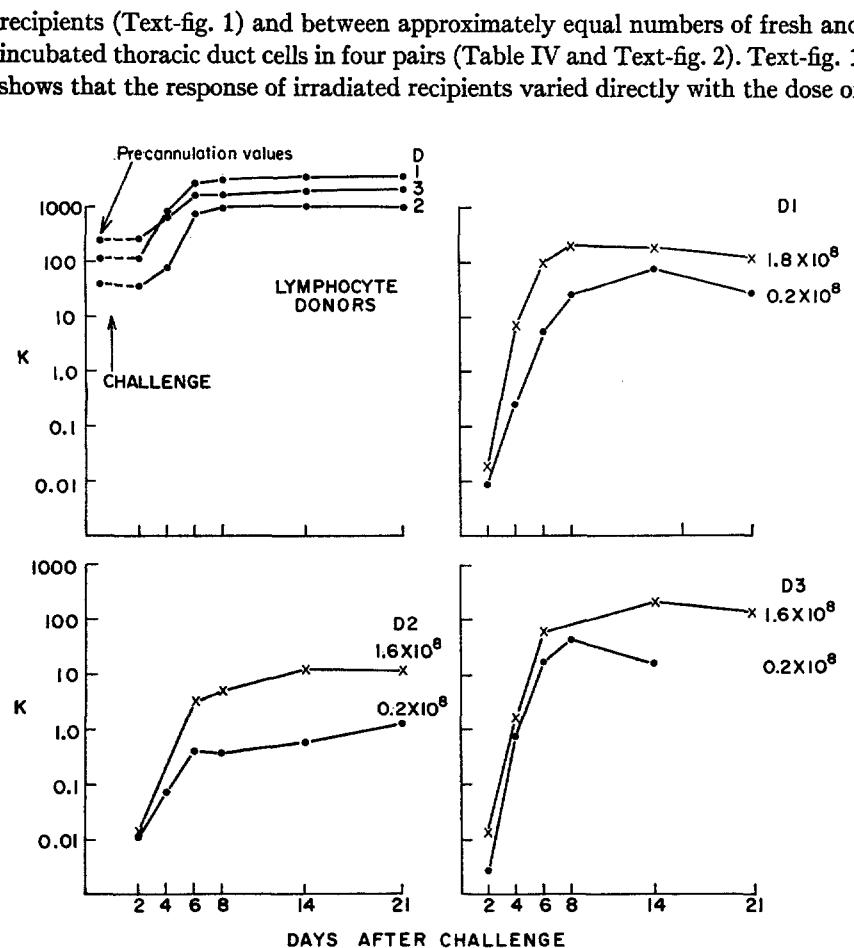
† Each recipient received 3 doses of cells from the same donor at 24, 38, and 50 hr after X-irradiation; 10^{10} ϕ X was added to the last dose of cells.

§ Donors challenged with 10^{10} ϕ X after 5 days' drainage from thoracic duct.

all the recipients to produce substantial amounts of antibody. In two pairs of rats a comparison was made between the effect of approximately equal numbers of fresh and incubated thoracic duct cells and in each case the recipient of the incubated cells gave a strikingly higher response.

Drainage of lymphocytes from the thoracic duct of two of the three immunized donors was continued for 5 days after which the fistulae were closed and the animals challenged with 10^{10} ϕ X intravenously. Table III shows that these lymphocyte-depleted rats still gave substantial secondary responses.

Cell Transfer 3 Months after Immunization.—A more extensive study was made of the ability of lymphocytes from rats immunized 3 months previously to confer secondary-type reactivity on X-irradiated recipients. Comparisons were made between the responses at two levels of cell dosage in three pairs of



TEXT-FIG. 1. Response of X-irradiated rats given $10^{10} \phi X$ and "fresh" thoracic duct cells at two levels of cell dosage from donors (D1-3) primarily immunized 3 months previously. Each member of a pair of recipients received a single dose of cells from the same donor together with $10^{10} \phi X$ 24 hr after X-irradiation. Cell dosage and identity of donor given for each pair of recipients. After 5 days' drainage from the thoracic duct the donors were themselves challenged with $10^{10} \phi X$ (top left).

cells administered while Table IV and Text-fig. 2 illustrate again the strikingly increased responsiveness of those recipients which were challenged with ϕX after receiving incubated thoracic duct cells, that is, inocula consisting almost exclusively of small lymphocytes. Incubated lymphocytes not only conferred higher responses when compared with equivalent numbers of fresh cells but they also led to a more rapid rate of antibody synthesis in the recipients. Ap-

proximate values for the time taken to double the level of serum antibody were calculated from the k values for the first 6 days after challenge and Table IV shows that these were shorter in the recipients of incubated cells.

The immunized donors which had been depleted of lymphocytes by drainage from the thoracic duct for 5 days gave high levels of antibody after challenge (Text-fig. 1). The peak values were of the same order as those in X-irradiated

TABLE IV
Comparison of Antibody Responses in X-Irradiated Rats Given 10^{10} ϕ X and either "Fresh" or "Incubated" Thoracic Duct Cells from Donors Immunized 3 Months Previously

Recipient No.*	Donor lymphocytes		Serum antibody (k) (days after challenge)†			Approximate doubling time‡	Donor No.
	i.v. dose ($\times 10^6$)	Status§	2	4	6		
5	2.5	f	0.016	—	3.5	14	
6	1.7	i	0.004	2.9	96	6	D2
8	1.6	f	0.014	1.9	63	7.5	
9	1.8	i	0.016	25	480	5.5	D3
11	2.5	f	0.27	0.5	13	19	
14	1.7	i	0.002	—	33	7.5	D2
12	2.3	f	1.6	4.6	96	16	
15	2.3	i	—	5.8	300	11	D3

* Each member of a pair of recipients received cells from the same donor. Recipients 5 to 9 were given a single dose of cells and 10^{10} ϕ X 24 hr after X-irradiation. Recipients 11 to 15 were given cells at 24, 37, and 49 hr after X-irradiation; 10^{10} ϕ X was added to last dose of cells.

† The responses of these rats beyond 6 days is shown in Text-fig. 2.

§ f, "fresh"; and i, "incubated" thoracic duct cells.

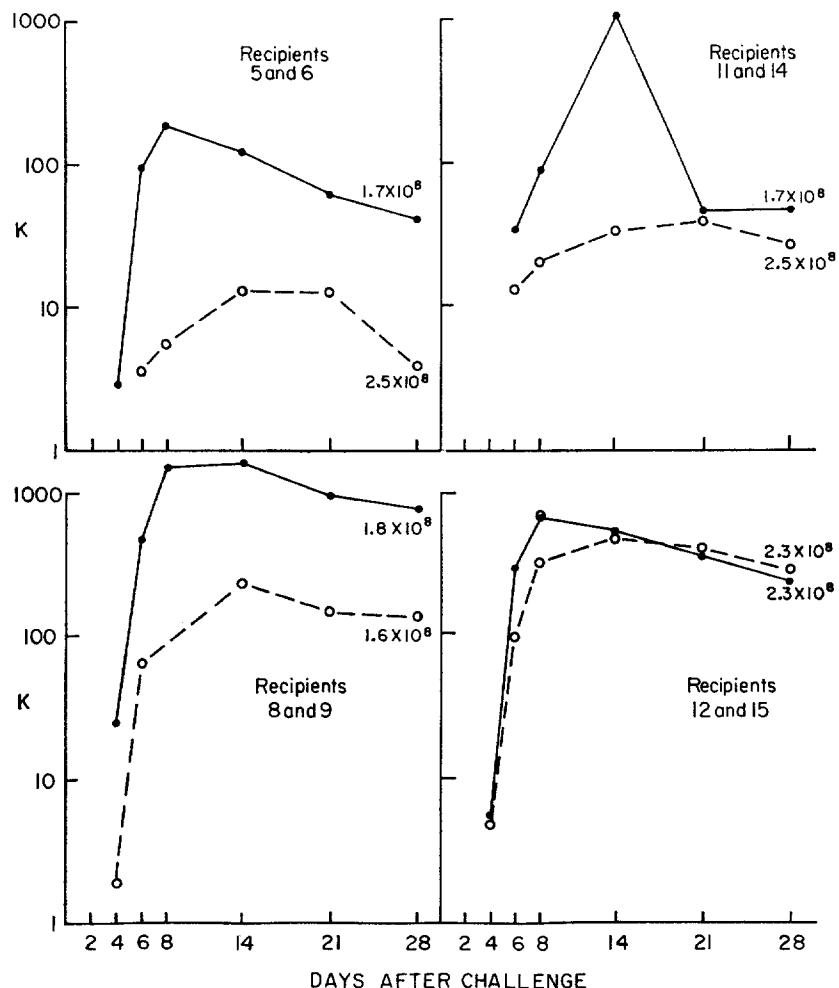
‡ Calculated from k values at 2, 4, and 6 days.

recipients of incubated thoracic duct cells (Text-fig. 2), but the total amount of antibody synthesized was much greater.

Cell Transfer 15 Months after Immunization.—The results of an experiment with fresh thoracic duct cells from a rat immunized 15 months previously are shown in Table V. Two important additional controls were included in this experiment: (a) the transfer of immune cells to irradiated recipients which were not subsequently challenged with ϕ X; and (b) the transfer of cells from a non-immunized donor to an irradiated recipient which was then challenged with ϕ X.

The results were similar in all respects to those obtained in the previous cell transfer experiments. In addition, it was shown that thoracic duct cells which

were obtained from a rat 15 months after primary immunization yielded negligible amounts of antibody after transfer unless the recipients were challenged with ϕX ; and that the antibody responsiveness conferred on X-irradiated recipients by nonimmune cells was very small in comparison to that given by immune cells, the peak titer being lower by a factor of 10^4 . Table V also shows that



Text-FIG. 2. Comparisons of responses of X-irradiated rats given $10^{10} \phi X$ and approximately equal numbers of either "fresh" (----○) or "incubated" (—●) thoracic duct cells from donors (D2 and 3 in Text-FIG. 1 and Table IV) primarily immunized 3 months previously. Incubated inocula (i.e. those lacking large lymphocytes) gave higher titers. Individual recipients can be identified by numbers in Table IV where experimental details are given.

the responsiveness to ϕX conferred by the transfer of only 80 million lymphocytes from the immunized donor rivalled in magnitude the secondary responses which were elicited from the donor after lymphocyte depletion, and from a non-cannulated rat which had also been primarily immunized 15 months previously.

To confirm that cell transfer without antigenic challenge resulted in the production of very little antibody in the recipients, an additional control experiment was carried out using incubated cells from a donor immunized 1½ months previously. $1 \text{ to } 1.5 \times 10^8$ thoracic duct cells were transferred to each of 4 irradiated recipients but the 4th only was challenged with $10^{10} \phi X$. 8 days after trans-

TABLE V
The Effect of Challenge with $10^{10} \phi X$ on the Antibody Response of X-Irradiated Rats Given "Fresh" Thoracic Duct Cells from either a Nonimmunized Rat or a Rat Primarily Immunized 15 Months Previously

Recipient No.	Donor lymphocytes*		Challenge with $10^{10} \phi X$ i.v.	Serum antibody (k)	
	i.v. dose ($\times 10^8$)	Status		Precannulation	8 days after challenge or cell transfer
1	1.0	Immune	None	—	0.022
2	0.8	Immune	+	—	110
3	1.0	Normal‡	+	—	0.018
4	1.0	Immune	None	—	0.004
5	None	—	+	—	0.01
Immune donor drained from thoracic duct for 5 days§.....			+	6.6	220
Normal (noncannulated) immune rat§.....			+	13	530

* Cells were given as a single dose, 24 hr after X-irradiation; ϕX , when given, was added to the cell dose.

† Cells from the thoracic duct of a normal, nonimmunized donor.

‡ Each rat immunized with $10^{11} \phi X$ 15 months previously.

fer, sera from the recipients had k values of 0.23, 0.12, and 1.27 for the 3 non-challenged animals and 92 for the recipient that received ϕX .

These results make it clear that the secondary-type responsiveness which was transferred to the irradiated recipients was due to cells carrying immunological memory; that is, to cells making extremely little antibody at the time of transfer but which responded vigorously after challenge with ϕX .

DISCUSSION

It has been shown that cells from the thoracic duct of primarily immunized rats can confer on X-irradiated syngeneic recipients the ability to respond to a first injection of antigen in a secondary-type manner. Thus, when cells were transferred 1½, 2, 3, or 15 months after a single immunizing dose of ϕX , all the

recipients responded to antigenic challenge with a rapid production of antibody. Negligible amounts of antibody appeared after such transfers if the recipients were not challenged; or if the recipients were challenged after receiving lymphocytes from a nonimmunized donor. This shows that lymphocytes from primarily immunized donors can mount a substantial secondary response if, but only if, they are challenged with antigen. Vredevoe and Hildemann (28) also noted that negligible amounts of antibody appeared in mice which had received lymphocytes from immunized donors, but they did not determine the effect of antigenic challenge on the recipients. The present experiments, which demonstrate clearly the existence of cells carrying immunological memory, differ in design from those of Vredevoe and Hildemann (28) and also from many others showing that lymphoid tissue which is actively synthesizing antibody continues to do so in adoptively immunized hosts without further challenge (17).

It was important to determine whether the immunological memory which was transferred to the recipients was carried by small or large lymphocytes because it has been claimed that antibody-forming cells in secondary responses arise exclusively from cells which are already dividing in the animal before antigenic challenge (18). To answer this question, the kinetics of the serum antibody response were compared in pairs of recipients which received from the same donor either fresh thoracic duct cells or incubated thoracic duct cells in which up to a 100-fold reduction in the number of large, dividing lymphocytes had been achieved before transfer. In all such comparisons, a reduction in the number of dividing cells did not reduce the power of the inocula to transfer secondary-type reactivity; indeed, unexpectedly, the rates of synthesis of antibody were increased and the peak concentrations of antibody were higher when incubated thoracic duct cells were employed. If the minute contaminating fraction of large lymphocytes had been responsible for the formation of antibody in the recipients of incubated cells, then the peak titers should have been achieved more slowly than in the rats receiving fresh inocula. In addition, it was shown that 8- to 9-fold differences between the number of fresh cells transferred were reflected by generally proportional differences in the absolute rates and peak titers of antibody formation. These experiments therefore, clearly point to the small, nondividing lymphocyte in thoracic duct lymph as the carrier of memory.

No satisfactory explanation can be given for the increased reactivity of thoracic duct cells which have undergone a period of incubation *in vitro*. An adjuvant effect by cell debris and the homing of incubated cells to sites in the recipient more favorable for the generation of an immune response are considered unlikely. The differential survival of a group of specifically reactive cells might have contributed marginally to the increased formation of antibody but it is thought that the major effect must have been due to changes in the responsiveness of individual reactive small lymphocytes. Such changes might have involved an increased ability to admit and retain antigen or an influence which

led to more rapid differentiation and cell division in response to antigen. In the absence of any experimental data to decide among these possibilities, it would be unprofitable to speculate further.

The small lymphocytes which have been shown in the present experiments to carry immunological memory to ϕ X were presumably formed as a result of primary immunization and later gave rise to antibody-forming cells during the secondary response by first differentiating into dividing precursors. An increase in the rate of formation of small lymphocytes has been demonstrated radioautographically by Nossal and Mäkelä (4) and by Miller (19) during immunization of rats with *S. adelaide*, but it has yet to be shown that such small lymphocytes evolve into antibody-forming cells during secondary responses.

The claim that small lymphocytes carry immunological memory to ϕ X in rats does not conflict with the demonstration that the lymphocyte-depleted donors yielded high levels of antibody after secondary challenge, nor with the previous finding that secondary responses to both sheep erythrocytes and tetanus toxoid could be elicited in immunized rats after lymphocyte depletion (14). Secondary responses in such animals may also be mediated by small lymphocytes since drainage from the thoracic duct for 5 days does not deplete the animal of all its small lymphocytes; some remain in lymphoid tissue and may not normally enter the recirculating pool (20). The peak concentrations of antibody achieved in the transfer experiments (Text-fig. 2) were obtained with relatively few cells (about 10% of the total number which can be collected during 5 days' drainage from the thoracic duct (14)), so it is not implausible to suggest that residual small lymphocytes might have accounted for the reactivity of the donors. Another possibility is that the response after lymphocyte-depletion was mediated by the cells composing germinal centers which have been implicated by Thorbecke et al. (21) as the generative compartments from which antibody-forming cells arise during secondary responses. Indeed, it is possible that two different cellular mechanisms may underlie secondary antibody responses: a short-term mechanism involving germinal centers and a long-term mechanism mediated by long-lived small lymphocytes. Studies on the sensitivity of secondary responses to X-irradiation support the idea of two different mechanisms following each other in sequence after primary immunization (22, 23). The relative radioresistance of the earlier phase could be interpreted as immunological memory invested in a dividing cell line in which repair might be possible after irradiation; and the radiosensitivity of the later phase might indicate investment in long-lived small lymphocytes.

The small lymphocytes in thoracic duct lymph which have been shown to carry immunological memory are long-lived cells (24, 25) which recirculate continuously from blood to lymph through the lymph nodes (20). It has been suggested that the process of lymphocyte recirculation may contribute to the efficiency of immune responses *in vivo* by making available to regionally stimu-

lated lymphoid tissue potentially reactive cells from the total recirculating pool; in this way cells eligible for induction could be recruited from the blood into a regional response as long as an appropriate local concentration of antigen persisted (9, 11). It has been shown that the primary immunological response of X-irradiated (26) and normal (27) lymphoid tissue can be augmented by a recruitment of lymphocytes from the blood and it will be important to determine if a similar process operates during secondary responses.

The demonstration that small lymphocytes carry immunological memory must be reconciled with the evidence that primary responses may also be initiated by small lymphocytes (7, 9). Any speculations about the properties of these two classes of small lymphocytes will beg fundamental questions about the nature of immunological commitment, but the simplest hypothesis is that primary and secondary responses result from the interaction of antigen with small lymphocytes possessing identical properties and that the immune animal has merely acquired many more specifically reactive cells as a consequence of cell division.

SUMMARY

Lymphocytes were obtained from the thoracic duct of rats $1\frac{1}{2}$ to 15 months after primary immunization with a single dose of bacteriophage ϕ X 174. An intravenous injection of these lymphocytes conferred on heavily X-irradiated rats the ability to form antibody in a secondary-type manner after a first injection of ϕ X. Negligible responses were obtained after cell transfer if the recipients were not challenged with antigen.

Thoracic duct cells from some immunized donors were incubated in vitro for 24 hr before transfer in order to destroy selectively the large, dividing lymphocytes. The responsiveness conferred on X-irradiated recipients by such "incubated" inocula was then compared with that given by equal numbers of "fresh" thoracic duct cells. In all such comparisons the recipients of the "incubated" cells gave higher and more rapid antibody responses. It was concluded that the cells in thoracic duct lymph which carried immunological memory were small lymphocytes.

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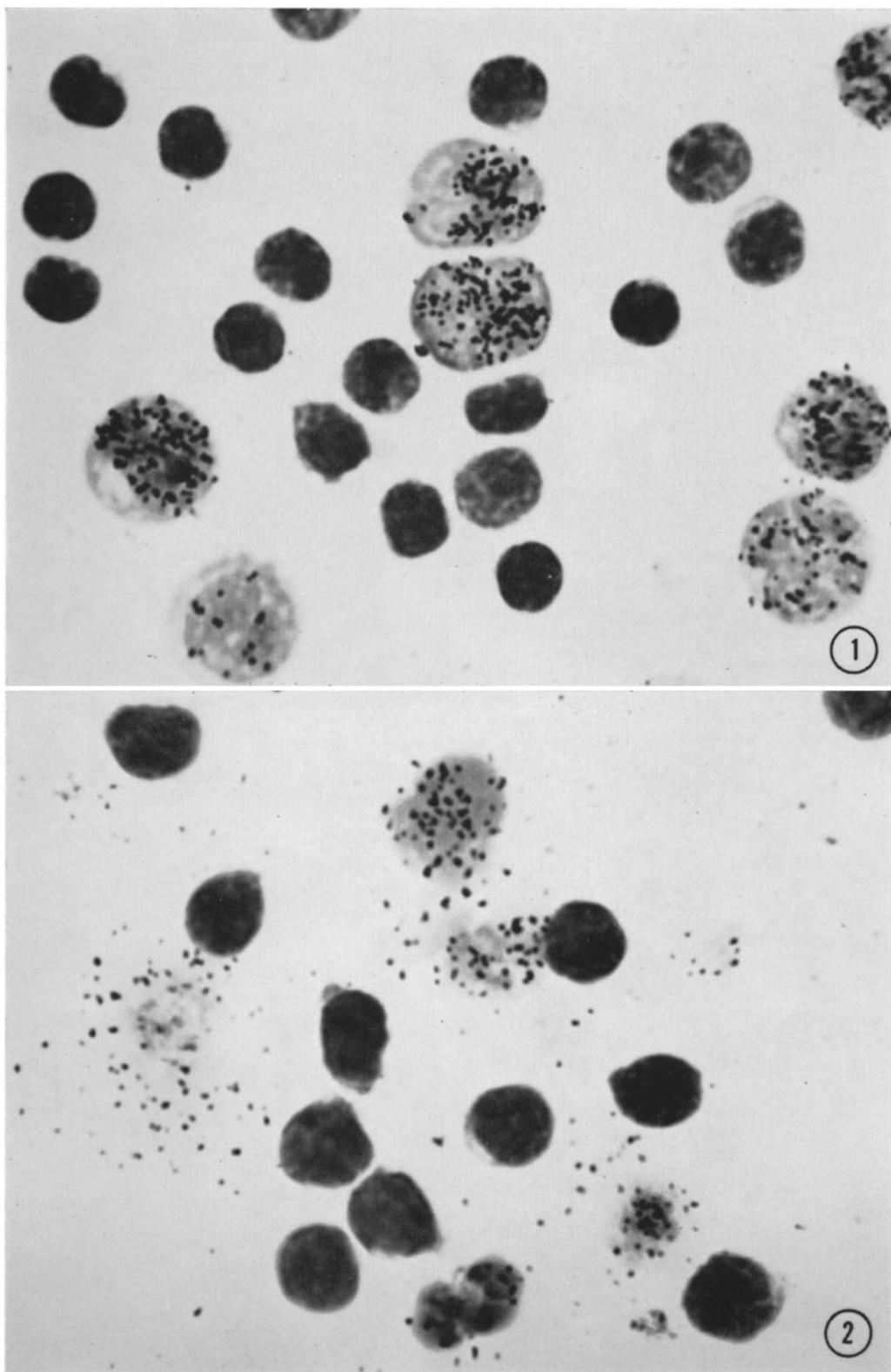
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EXPLANATION OF PLATE 96

FIG. 1. Radioautograph of thoracic duct lymphocytes in second 12 hr collection of lymph from rat which had received a continuous intravenous infusion of tritiated thymidine. All the large and medium lymphocytes are labeled (Experiment 1 in Table I). Exposure 28 days. $\times 2000$.

FIG. 2. Radioautograph of sample of cells shown in Fig. 1 after incubation in vitro at 37°C for 24 hr. Labeled debris shows selective destruction of large and medium lymphocytes (see Table I). Exposure 28 days. $\times 2000$.



(Gowans and Uhr: Immunological memory)