Immunology, 1968, **14**, 621.

Graft *versus* Host Reaction in Tissue Culture

I. LYSIS OF MONOLAYERS OF EMBRYO MOUSE CELLS FROM STRAINS DIFFERING IN THE H-2 HISTOCOMPATIBILITY LOCUS BY RAT LYMPHOCYTES SENSITIZED *IN VITRO*

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(*Received 2nd August* 1967)

Summary. Rat lymphocytes cultured on mouse embryo cell monolayers produced large pyroninophilic cells (LPC) which lysed the mouse cells. The LPC that developed on monolayers of any particular strain of mouse (originator monolayers) were tested, by transfer, for their ability to lyse monolayers of other mouse strains. The distribution of lysis among the various strains of mouse revealed a definite pattern of specificity. Analysis of the H-2 allelic complement of the mouse strains tested suggests that the lymphocytes were sensitized upon exposure to the mouse embryo monolayers against one or more of the antigens determined by the H-2 locus. The presence or absence of one or all of the antigens in other strains determined whether monolayers of these strains were lysed completely, partially, or not at all. It was concluded that the cultures obtained are an *in vitro* reflection of the graft *versus* host immune reaction. It was produced in the tissue culture as a primary response by normal lymphocytes.

INTRODUCTION

When suspensions of lymphocytes prepared from lymph nodes or the thoracic duct fluid of the rat are grown on mouse embryo cell monolayers or on embryo monolayers of other strains of rats, large lymphoid cells, with strongly pyroninophilic cytoplasm, appear in the cultures during the first 4–5 days. A lytic process affecting the embryonic fibroblasts occurs subsequently (Ginsburg and Sachs, 1965; Ginsburg, 1965). The early large pyroninophilic cells (LPC) (Gowans, 1962) that appear on the 2nd and 3rd days are derived from the small lymphocytes, as was demonstrated by [^{3}H]thymidine-labelling of the small lymphocytes (Ginsburg, Tyler and Everett (1967). Subsequently, the LPC increase considerably in number by mitosis, and a fairly pure culture, composed of many millions of LPC growing mostly in suspension, is obtained on the 7th to 10th day of the culture (Ginsburg, 1965; Ginsburg and Sachs, 1965). It was shown that this type of culture is obtained on xenogeneic mouse monolayers and on allogeneic monolayers of other strains of rat (Ginsburg, 1965). On syngeneic monolayers, entirely different types of culture were produced (Ginsburg, 1965). It is interesting to note that attempts to obtain the lytic effect with mouse lymphocytes were unsuccessful, either mast cells or histiocytes being produced in the cultures (Ginsburg and Lagunoff, 1967).

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In the present study the ability of LPC produced on a monolayer of a mouse strain to lyse monolayers of other strains has been investigated in order to determine the specificity of the lytic reaction. The lytic effect has been found to be a response of the graft *versus* host reaction type, developed in tissue culture by unsensitized normal rat lymphocytes. The lytic ability of the LPC shows a specificity to the allelic antigens determined by the H-2 locus of the mouse. The lymphocytes were thus sensitized *in vitro* against these mouse tissue transplantation antigens. The resulting LPC were not able to lyse mouse embryo monolayers of other strains, which did not contain the same antigenic alleles.

MATERIALS AND METHODS

Nomenclature

The type of culture in which the embryo cell monolayers were lysed by LPC will be termed graft reaction cultures, to distinguish them from other types in which histiocytic–lymphocytic or mast cell reactions supervened (Ginsburg, 1965; Ginsburg and Lagunoff, 1967).

A batch of monolayer denotes a culture of embryo cells prepared as a primary from one (or, very infrequently, two) litters, and successively passaged by trypsinization. The batches of monolayer on to which the rat lymphocytic cell suspensions are plated for the first time and on which the LPC develops will be termed originator monolayers.

Animals

Donor rats for lymph node cells were of inbred Lewis and Brown–Norway (BN), and random bred Sprague–Dawley (SD) and Wistar strains, both males and females, weighing 150–300 g. In three experiments, lymph node cells from BN failed to produce graft reaction cultures. Suspensions of lymph nodes from SD and Wistar rats were prepared from single animals whereas those from Lewis rats were prepared from pools of cells from several animals.

Embryo cell monolayers were obtained from the following mouse strains: A/He(H-2^a); C57BL/6J, SWR/J(H-2^b); BALB/CAn, BALB/CJ, BALB/CCu, DBA/2An(H-2^d); C3H/He, C3H/J, AKR/Cu(H-2^k); F_1(C3H × C57BL/6) and F_1(C57BL/6 × BALB/C). From each strain, several batches of monolayers from litters of embryos 6–15 mm long were prepared. Some of the monolayer batches could be passaged at intervals over a period of many months.

Embryo monolayers from rat strains SD, Wistar, Lewis and BN made from embryos 10–20 mm long, supported rat lymphoid cell growth very well (Ginsburg, 1965; Ginsburg and Sachs, 1965). Human skin explant propagated in culture was also used in some experiments.

Tissue culture

Tissue culture procedures were carried out as described previously (Ginsburg and Sachs, 1965; Ginsburg, 1965).

(a) *Medium*: Waymouth's medium (Waymouth, 1959), supplemented with 10 per cent calf serum, was used to establish the monolayers. In order to maintain them, 0·5 per cent lactalbumin hydrolyzate in Earle's saline with 10 per cent calf serum was used. For lymphoid cell culture, Dulbecco's modification of Eagle's medium was used, with the addition of 20 per cent horse serum as described previously (Ginsburg, 1965; Table 2)*.

* The description of Dulbecco's medium reported in this table is misprinted: L-threonine, 9·52 g should have been included, and 6·60 g L-phenylalanine was used, not 9·52 g.

(b) *Monolayers*: Embryo cell monolayers were prepared, stored and used as described previously (Ginsburg, 1965; Ginsburg and Sachs, 1965). In general, the lymphoid cells were plated on secondary and tertiary cultures, but further passages up to the eighth have been successfully used. In some cases, passages of monolayers incubated for periods of up to 70 days originated good graft reaction cultures.

(c) *Lymph node cultures*: Cultures of lymph node cells were prepared and maintained as previously described (Ginsburg and Sachs, 1965; Ginsburg, 1965).

Lymph node cells, $1{\cdot}5–4\times10^7$ (usually 3×10^7), were seeded on monolayers in 60-mm plastic Petri dishes. The cultures were incubated at 37° in a humidified incubator with an inflow of 7 per cent CO_2 in air.

(d) *Transfer of the lymphoid cells*: The cultures were incubated for 4–6 days, after which time there was already an appreciable quantity of LPC and the first signs of lysis could be seen (Ginsburg and Sachs, 1965). The free-floating cells were collected by pipetting. The large cells, which presumably included all the LPC engaged in the lytic reaction, and the small cells, which were predominantly lymphocytes, were counted separately. The cell types were easily distinguishable by their distinct size difference; cells of the size of the LPC are entirely absent in the first 2 days of culture. The cell suspensions were centrifuged at 800 rev/min in an International Clinical centrifuge, Head No. 215, for 7 minutes, resuspended in fresh Dulbecco's medium with 20 per cent horse serum and distributed among monolayer plates of several different strains.

Evaluation of the lytic effect

In early experiments, the rate of lysis was recorded by observing the plates 2–3 hours after the transfer and twice a day thereafter. Lytic activity was estimated on the basis of the area of the monolayer lysed and the time taken for complete lysis. The degree of lysis was expressed semiquantitatively: (++++) indicates complete lysis; (+++) = 75 per cent of the monolayer; (++) = 50 per cent; (+) = 25 per cent; (±) = very weak signs of lysis on the threshold of identification, and (−) = no detectable lytic effect.

In later experiments, lysis was quantified as follows: 2 ml of Dulbecco's medium containing 0·0025 per cent Neutral Red was added to plates. Within a few hours, the dye was markedly localized in the cytoplasm of all the fibroblasts and faintly in some of the lymphoid cells. After 24 hours, the medium from the plates was harvested, 5 ml of 0·25 per cent trypsin was added and the plates were incubated for 15 minutes at 37°. The cells of the monolayer were dispersed by pipetting, collected and added to the medium. A suspension was thus obtained composed of only single cells of two cell categories, the fibroblasts and the lymphoid cells, which were easily distinguishable as the fibroblasts were considerably larger than the lymphoid cells. Their cytoplasm and cell surface were coarse in appearance and stained particles or vacuoles were abundantly scattered over the cytoplasm and encircled the nucleus. The lymphoid cells had a homogeneous cytoplasm and smooth cell surface, and the red stain, if present, was confined only to several red dots or vacuoles, in the Golgi zone. Macrophages were also distinguished by deep and complete staining of the cytoplasm. In each suspension, only the fibroblasts were counted in a haemocytometer. Three to seven counts on each sample gave the mean number of fibroblasts. The same results were obtained when counting two to four plates of each group separately or when pooling the plates before counting. The standard deviation calculated from the mean obtained from several plates was found to be not greater than the standard deviation of the haemocytometer counts. In other experiments, two plates were pooled for

counting and two other plates were fixed and stained. There was a direct correlation between the number of surviving fibroblasts and the area of lysis seen in the plates (see Table 5 and Fig. 1). Monolayers cultured in parallel to the experimental cultures but without lymphoid cells served as controls. It was found that a culture rated (−) or (±) might show

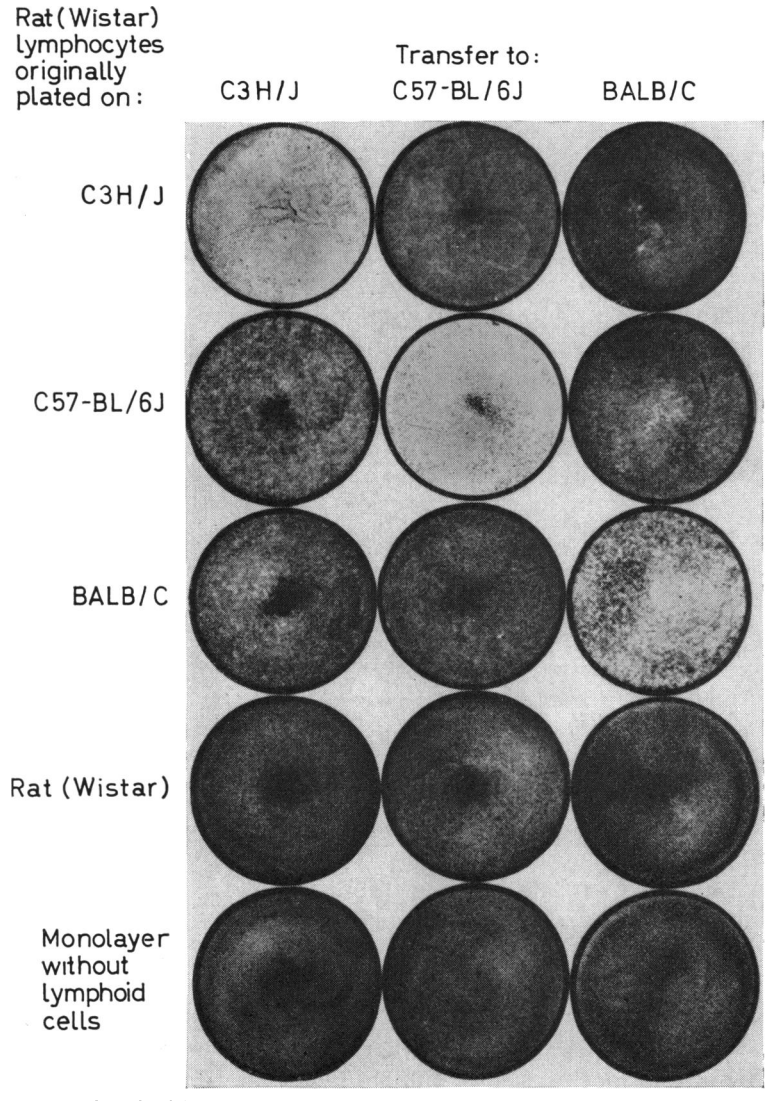

FIG. 1. Cross-transfer of LPC among monolayers of mouse strains differing in their H-2 locus. Plates were fixed 2 days after transfer. In the transfers from C3H to C3H and C57BL/6 to C57BL/6 no fibroblasts survived and the staining seen is contributed by the lymphoid cells adhering to the film (Figs. 3 and 5). Counts of surviving fibroblasts of other plates of the same transfer are shown in Table 5. May–Grünwald–Giemsa.

as much as 30 per cent less cells than the control, possibly because of invisible lysis or the effect on the mitosis of fibroblasts. After trypsinization, neither unstained fibroblasts nor any cells which could be stained with trypan blue were present, since trypsinization completely destroys dead cells.

Fixation and staining

Plates were twice washed with saline and fixed with absolute methanol. For observation of the monolayers, the plates were stained with May–Grünwald–Giemsa stain. In order to observe the lymphoid populations, the plates were stained with methyl green–pyronin as previously described (Ginsburg and Sachs, 1965).

RESULTS

GRAFT REACTION IN TRANSFER CULTURES OF RAT LPC FROM ORIGINATOR MONOLAYERS TO MONOLAYERS OF THE SAME STRAIN

The LPC donor plates consisted of 5th or 6th day cultures in which lysis had just appeared, or before its appearance. Part of these monolayers were left intact for subsequent determination of the rate of lysis; lymphoid cells were collected and transferred from the remaining parts. In a number of experiments in which LPC which had originated on one batch of BALB/C or on C3H were transferred to several different batches of BALB/C or C3H, respectively, complete or almost complete lysis of the fibroblasts always occurred. Extent of lysis of the originator batch was never consistently greater than that of the other batches. The amount of LPC harvested on the 5th or 6th days and the proportion of large to small cells varied from monolayer to monolayer, ranging in cultures with strong lytic activity from 1 to 5×10^6 large cells and 1 to 15×10^6 small ones. When such numbers of cells were plated onto a monolayer of the same strain, containing from 1 to $2{\cdot}5 \times 10^6$ fibroblasts, complete lysis could be obtained after only 19 hours. Less than 3 hours after plating, fibroblasts undergoing lysis were clearly observed, while at 6 hours there were numerous cells in various phases of the lytic process, and after 19–24 hours, not a single fibroblast survived (Fig. 5). Only a fibrous mesh remained (Figs. 3 and 5) (seen in living cultures as transparent film), to which many LPC, fragments of lysed cells and pyknotic bodies of the disintegrated cells of the lymphoid suspension adhered. The fibres could be detached and disrupted by slight movement of the Petri dish. The lysing fibroblasts acquired a characteristic shape which could easily be identified in the living cultures. These cells show cytoplasmic droplets budding peripherally in a rosette-like form (Fig. 2), until only masses of cytoplasmic particles remain (Fig. 2) (Ginsburg and Sachs, 1965; Ginsburg, 1965). The presence of such forms, film formation and increase in the number of cytoplasmic particles, as well as the disappearance of cells in the monolayer, was a clear indication of lysis which could easily be distinguished from other nonspecific effects caused sometimes by dense aggregates of lymphoid cells, detachment of fibroblasts or acquisition of fusiform shape by fibroblasts or development of cytoplasmic inclusions. Thus, detachment may occur when a large number of lymphoid cells, no matter of what origin, is plated on fibroblasts distributed very sparsely over the plate. To avoid such a detachment, which is due to the high motility of the lymphoid cells, the monolayer should consist of a continuous sheet with all the fibroblasts in contact with each other (see Figs. 4, 6 and 8).

When lysis culminated at 19–24 hours, LPC in excess of the number needed for complete lysis of the monolayer were present. On a second transfer of these LPC to a fresh monolayer, complete lysis occurred within 24 hours.

TRANSFER OF RAT LPC TO MONOLAYERS OF OTHER STRAINS

In these transfers, monolayers from strains that differed in the strong H-2 locus and monolayers from strains that did not differ in the H-2 locus but differed in other weaker

TABLE 1

LYSIS OF DIFFERENT MONOLAYERS BY TRANSFER OF RAT LPC ORIGINATED ON DIFFERENT MOUSE STRAIN MONOLAYERS

Rat (SD) lymphocytes originally plated on monolayers*	No. of large (LC) and small cells (SC) transferred per new plate ($\times 10^6$)	Lymphoid cells transferred to monolayers											
		BALB/CAn (H-2^d)		DBA/2An (H-2^d)		C57BL/6An (H-2^b)		C3H/He (H-2^k)		SD		Human skin line	
		Extent†	Time†	Extent	Time	Extent	Time	Extent	Time	Extent	Time	Extent	Time
BALB/CAn	11·2 LC 16·3 SC	+++	96	+++	96	±	96	–	96	–	96	–	96
BALB/CJ	–			++++	48								
BALB/CAn	–	+++	72	++++	48								
DBA/2An	–	+++	72	++++	48								
BALB/CAn	2·3 LC 6·3 SC	+++	96	++++	48								
C3H/He	1·3 LC 5·8 SC	++	96			±	96	++++	48				
Via C57BL/6‡	3.3 LC 7·2 SC					+	72	++++	33				
C57BL/6An	2·0 LC 9·5 SC	+++	96			++++	48	–	96				
Via C3H§	4·8 LC 12·0 SC					++++	33	–	72				

* Cultures of lymph nodes were prepared separately from three different rats at the same time and on the same monolayers.

† Extent of lysis and time to complete lysis or final reading when no further lysis occurred (hours).

‡ LPC originated on C3H/He and transferred to C57BL/6An were retransferred from part of the C57BL/6An monolayers 48 hours later back to C3H and again to C57BL/6An.

§ LPC originated on C57BL/6An and transferred to C3H/He monolayers were retransferred from part of the C3H monolayers 48 hours later back to C57BL/6An and again to C3H/He.

histocompatibility loci were employed. Some of the many experiments performed are illustrated in Tables 1–4. LPC transferred to monolayers of strains that did not differ in the H-2 locus all lysed these strains to the same extent as the originator strain, e.g. transfers from monolayer of BALB/C(H-2^d) to DBA_2(H-2^d) or vice versa (Tables 1 and 2), or from AKR(H-2^k) to C3H (Tables 3 and 4) and to F_1(C3H × C57BL/6) (Table 4). In contrast, transfers to monolayers of other H-2 groups, e.g. from BALB/C (H-2^d) to C3H(H-2^k) or to C57BL/6(H-2^b) (Table 1) or from C3H(H-2^k) to C57BL/6(H-2^b) and vice versa (Tables 1 and 2) resulted in either slow or partial lysis, or no lysis at all. Similar results were obtained when LPC originated on monolayers of one H-2 group were transferred to monolayers of two different strains both belonging to the second H-2 group (Table 3). A clear difference in lytic ability was demonstrated when LPC originated on C57BL/6 were

TABLE 2

LYSIS OF DIFFERENT MONOLAYERS BY TRANSFER OF RAT LPC ORIGINATED ON DIFFERENT MOUSE AND RAT MONOLAYERS

Rat (SD) lymphocytes originally plated on monolayers	No. of large (LC) and small cells (SC) transferred per new plate (× 10^6)	Lymphoid cells transferred to monolayers									
		C3H/He (H-2^k)		C57BL/6An (H-2^b)		DBA/2An (H-2^d)		BN		Lewis	
		Extent*	Time*	Extent	Time	Extent	Time	Extent	Time	Extent	Time
C3H/He	1·3 LC 9·0 SC	++++	48	−	120						
Via C57BL†	3·3 LC 9·0 SC	++++	24	−	72						
BALB/CAn (H-2^d)	2·2 LC 5·0 SC	+++	120			++++	48				
Via C3H‡	4.6 LC 6·2 SC	++++	72			++++	19				
BN	1·0 LC 9.0 SC	−	96					+	96	−	96

* Extent of lysis and time to complete lysis or final reading when no further lysis occurred (hours).

† LPC originated on C3H/He and transferred to C57BL/6An monolayers were retransferred from part of the C57BL/6An monolayers 48 hours later back to C3H/He and again to C57BL/6An.

‡ LPC originated on BALB/CAn and transferred to C3H/He monolayer were retransferred from part of the C3H/He monolayers 48 hours later to DBA/2An and again to C3H/He.

transferred to originator, to F_1(C57BL/6 × BALB/C) and to BALB/C monolayers (Table 3). While both C57BL/6 and F_1(C57BL/6 × BALB/C) were completely lysed within 72 hours, the monolayer of BALB/C remained intact.

In order to test whether lack of lysis on transfers to monolayers of other H-2 groups might be due to loss of lytic ability of the LPC, because of undesirable conditions relating to the tissue culture or for any other reasons, the lymphoid cells from part of the plates of such transfer cultures were retransferred back to the originator monolayer and again to the same monolayer of the first transfer. As shown (Tables 1 and 2) in back transfers the originator monolayers were lysed completely, even faster than on the first transfer. It should be noted that after transfers performed on the 5th or 6th day, the increase of LPC by mitosis is extremely rapid (Ginsburg, unpublished).

TABLE 3

TRANSFER TO DIFFERENT MOUSE CELL MONOLAYERS OF RAT LPC ORIGINATED ON TWO DIFFERENT MOUSE STRAIN MONOLAYERS

Rat (Lewis) lymphocytes originally plated on monolayers	No. of large (LC) and small cells (SC) transferred per new plate ($\times 10^6$)	Lymphoid cells transferred to monolayers											
		AKR ($H\text{-}2^k$)		C3H/J ($H\text{-}2^k$)		C57BL/6J ($H\text{-}2^b$)		SWR/J ($H\text{-}2^b$)		BALB/C ($H\text{-}2^d$)		F_1(C57BL/6J × (BALB/C)	
		Extent*	Time*	Extent	Time	Extent	Time	Extent	Time	Extent	Time	Extent	Time
AKR†	3·5 LC 5·6 SC	++++	48	++++	32	–	96	–	96				
C57BL/6J†	1·7 LC 1·8 SC	±	96	±	96	++++	32	++	96				
C57BL/6J‡	–					++++	72			–	72	++++	72

* Extent of lysis and time to complete lysis or final reading when no further lysis occurred (hours).
† LPC transferred on the 5th day.
‡ LPC transferred on the 8th day.

TABLE 4

SURVIVAL OF FIBROBLASTS OF $H\text{-}2^k$ AND $H\text{-}2^d$ GROUPS 6 DAYS AFTER TRANSFER OF LPC ORIGINATED ON AKR($H\text{-}2^k$)

Rat (Wistar) lymphocytes originally plated on:	No. of lymphoid cells transferred per new plate ($\times 10^6$)	Lymphoid cells transferred to monolayers							
		AKR ($H\text{-}2^k$)		C3H/J ($H\text{-}2^k$)		F_1(C3H × C57BL/6)		BALB/C ($H\text{-}2^d$)	
		No. cells surviving ($\times 10^6$)	%	No. cells surviving ($\times 10^6$)	%	No. cells surviving ($\times 10^6$)	%	No. cells surviving ($\times 10^6$)	%
AKR	3·80	0·258	27	0·268	10·8	0·462	21·3	1·262	70·2
Control monolayer without lymphoid cells	–	0·946	–	2·500	–	2·162	–	1·745	–

TABLE 5

SURVIVAL OF FIBROBLASTS OF THREE MOUSE STRAINS 2 DAYS AFTER TRANSFER OF LPC ORIGINATED ON FOUR DIFFERENT MONOLAYERS*

Rat (Wistar) lymphocytes originally plated on:	No. large (LC) and small cells (SC) transferred per new plate ($\times 10^6$)	Lymphoid cells transferred to monolayers†					
		C3H/J		C57BL/6J		BALB/C	
		No. fibroblasts surviving ($\times 10^6$)	% survival‡	No. fibroblasts surviving ($\times 10^6$)	% survival‡	No. fibroblasts surviving ($\times 10^6$)	% survival‡
C3H/J	1·05 LC 1·66 SC	0·062	4·7	1·357	61·0	1·010	79·2
C57BL/6J	1·03 LC 2·24 SC	0·524	39·4	0·057	2·6	0·791	62·5
BALB/C	0·85 LC 1·40 SC	0·917	68·9	1·589	71·5	0·374	29·5
Wistar	0·40 LC 2·96 SC	1·242	93·4	1·804	81·1	1·183	93·7
Control monolayer without lymphoid cells	–	1·330	–	2·223	–	1·264	–

* See Fig. 1 of same experiment.
† Monolayers were prepared 1 day before the transfer of the lymphoid cells by plating $1{\cdot}2 \times 10^6$ fibroblasts/plate.
‡ Calculated from control monolayers without lymphoid cells.

TABLE 6

SURVIVAL OF FIBROBLASTS OF FOUR MOUSE STRAINS 3 DAYS AFTER TRANSFER OF LPC ORIGINATED ON MONOLAYERS OF THE FOUR STRAINS

Rat (Lewis) lymphocytes originally plated on:	No. large (LC) and small cells (SC) transferred per new plate ($\times 10^6$)	Lymphoid cells transferred to monolayers*							
		C3H/J		C57BL/6J		BALB/C		A/He	
		No. fibroblasts surviving ($\times 10^6$)	% survival†	No. fibroblasts surviving ($\times 10^6$)	% survival†	No. fibroblasts surviving ($\times 10^6$)	% survival†	No. fibroblasts surviving ($\times 10^6$)	% survival†
C3H/J	1·10 LC 7·60 SC	0·027	2·0	2·816	85·0	1·250	54·1	0·026	2·3
C57BL/6J	0·55 LC 6·25 SC	0·246	18·3	0·151	4·5	1·484	64·2	0·086	7·5
BALB/C	2·36 LC 7·40 SC	0·435	32·4	2·457	74·2	0·030	1·3	0·014	1·2
A/He	1·15 LC 4·70 SC	0·057	4·2	3·132	94·2	0·233	10·0	0·038	3·3
Control of monolayer without lymphoid cells	–	1·342	–	3·313	–	2·312	–	1·144	–

* Monolayers were prepared 2 days before the transfer of the lymphoid cells by plating 8×10^5 fibroblasts/plate.
† Calculated from control monolayers without lymphoid cells.

TABLE 7

H-2 LOCUS HISTOCOMPATIBILITY ALLELIC ANTIGENS SHARED BY STRAINS OF MOUSE USED IN EXPERIMENTS*

Strains	Alleles																		
	A	C	D	E	F	H	J	K	M	N	V	Y	A^1	B^1	C^1	D^b	D^k	E^d	K^b
BALB/C–C3H	–	C	–	–	–	H	–	–	–	–	–	–	–	–	–	–	–	–	–
BALB/C–C3H–A	–	C	–	–	–	H	–	–	–	–	–	–	–	–	–	–	–	–	–
BALB/C–A	–	C	D	–	F	H	J	–	M	N	–	–	A^1	B^1	C^1	–	–	–	–
BALB/C–C57BL/6	–	–	–	–	F	–	–	–	–	N	–	–	A^1	B^1	C^1	–	–	–	–
BALB/C–C57BL/6–A	–	–	–	–	F	–	–	–	–	N	–	–	A^1	B^1	C^1	–	–	–	–
BALB/C–C57BL/6–C3H	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C3H–C57BL/6	–	–	–	E	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C3H–C57BL/6–A	–	–	–	E	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C3H–A	A	C	–	E	–	H	–	K	–	–	–	Y	–	–	–	–	–	–	–
C57BL/6–A	–	–	–	E	F	–	–	–	–	N	–	–	A^1	B^1	C^1	–	–	–	–

Strains	Alleles not shared by other strains																		
	A	C	D	E	F	H	J	K	M	N	V	Y	A^1	B^1	C^1	D^b	D^k	E^d	K^b
BALB–A	–	–	D	–	–	–	J	–	M	–	–	–	–	–	–	–	–	–	–
C3H–A	A	–	–	–	–	–	–	K	–	–	–	Y	–	–	–	–	–	–	–
A($H\text{-}2^a$)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C57BL/6, SWR($H\text{-}2^b$)	–	–	–	–	–	–	–	–	–	–	V	–	–	–	–	D^b	–	–	K^b
BALB/C, DBA($H\text{-}2^d$)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	E^d	–
C3H, AKR($H\text{-}2^k$)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	D^k	–	–

* From Snell, Hoecker, Amos and Stimpfling (1964).

TESTS FOR THE SPECIFICITY OF THE LYTIC REACTION BY CROSS-TRANSFERS OF RAT LPC AMONG MONOLAYERS OF MOUSE STRAINS DIFFERING IN THEIR H-2 LOCUS

Ten experiments were performed involving monolayers of C57BL/6(H-2^b), BALB/C(H-2^d) and C3H(H-2^k) strains. In five of these experiments, the A/He(H-2^a) strain was also included. The results of three experiments are represented in Tables 1, 5 and 6 and in Figs. 1 and 3–8. The distribution of lysis among the different strains was variable from experiment to experiment, when different batches of monolayers of the same strains were used as originators. For example, in the experiment shown in Table 1, LPC which originated on C57BL/6J lysed readily BALB/CAn, but not C3H. However, in other experiments (Tables 5 and 6) with different batches of monolayers C3H was severely affected and BALB/C hardly so. Despite these inconsistencies, a clear pattern of specificity was apparent in the lytic effect, broadly in accordance with the distribution of the H-2 alleles of the strains tested. Table 7 shows H-2 histocompatibility alleles shared or unique to a given strain of mouse used in the present study. It is of particular interest that the three main strains used, BALB/C, C57BL/6 and C3H, do not have any of these antigens in common. If the LPC were sensitized against the tissue antigens determined by the H-2 locus, then LPC originated on one of these three strains, e.g. on C57BL/6, lysed completely, or nearly so, one of the other two, e.g. BALB/C, all the LPC or a large proportion of them would have been sensitized against one or more antigens that are present in BALB/C and absent in C3H. It might be expected, therefore, that the same LPC should be unable, or only weakly able, to lyse the third strain. Further, the minimal number of LPC required to lyse completely the originator strain will be unable to lyse completely monolayers of the two other strains with approximately the same number of fibroblasts. In fact, in all the experiments where a monolayer of a second strain was lysed vigorously, the third was not (Tables 1 and 6). There were cases in which both were not lysed or only partially lysed. Each of these strains has alleles which are either unique or are shared by the A strain (Table 7).

Table 7 shows that the A/He strain contains all those alleles that the other three strains share with each other. Therefore, A strain in any combination of lysis with those other three strains should be lysed either equally or more strongly than the other strains. The A strain could be lysed extensively by LPC originated on BALB/C or C3H, since each of these strains shares more alleles with A, but not by those LPC that had originated on C57/BL6 which does not share additional alleles. In accordance with this prediction, the A strain was repeatedly lysed completely by LPC originated on C3H or BALB/C and less strongly by LPC originated on C57BL/6, but to an extent which would correspond to the combined rate of lysis on C3H and on BALB/C (Table 6).

Furthermore, it should be impossible for LPC originated on the A/He strain to lyse completely more than two other strains. If both C3H and BALB/C were lysed completely and not C57BL/6, this would imply that the allele E was not involved, since, were it otherwise, if a certain proportion of the LPC lysed C57BL/6, this would be reflected in a lesser lysis in the BALB/C monolayer which lacks this allele. In the same way, if the alleles F, N, A^1, B^1 and C^1 are involved, C3H would display a lesser degree of lysis. Thus, there can be either complete lysis of one or two strains or partial lysis of all three but never complete lysis of all the three strains; this is shown by the experimental data (Table 6).

The present study, therefore, leads to the conclusion that rat LPC originated on mouse monolayers are, at most, specifically sensitized against the tissue transplantation antigens

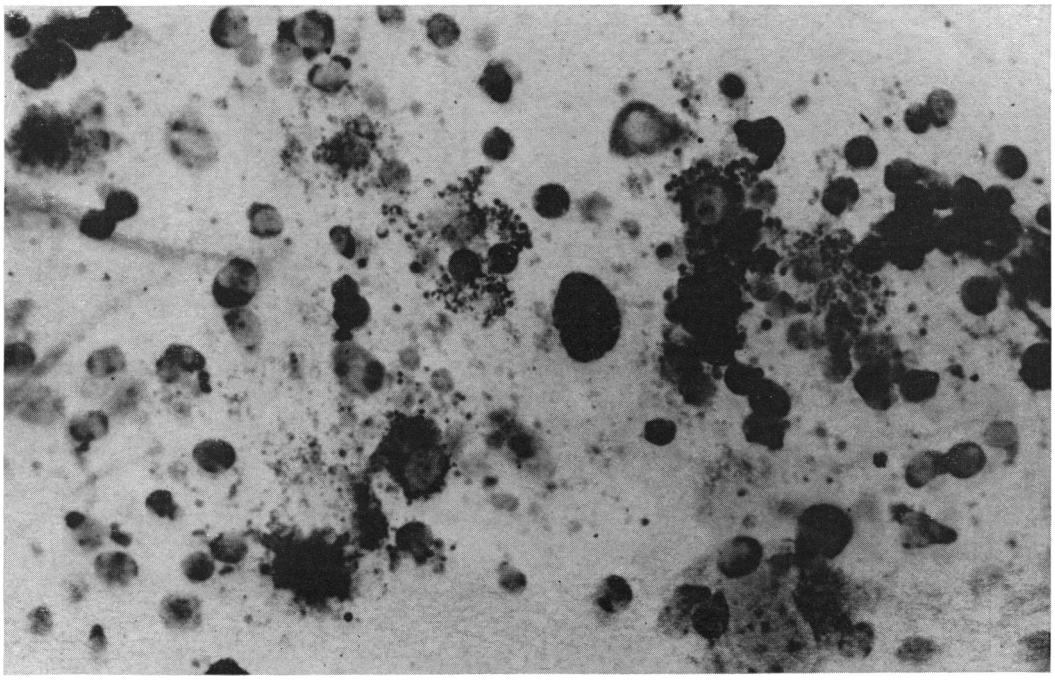

FIG. 2. Fibroblasts of C3H/J undergoing lysis by LPC of Sprague–Dawley rat. The fibroblasts split to cytoplasmic droplets. Two days after transfer of $1{\cdot}1 \times 10^6$ LPC originated on C3H monolayer. Methyl green–pyronin, × 400.

(*Facing p.* 632)

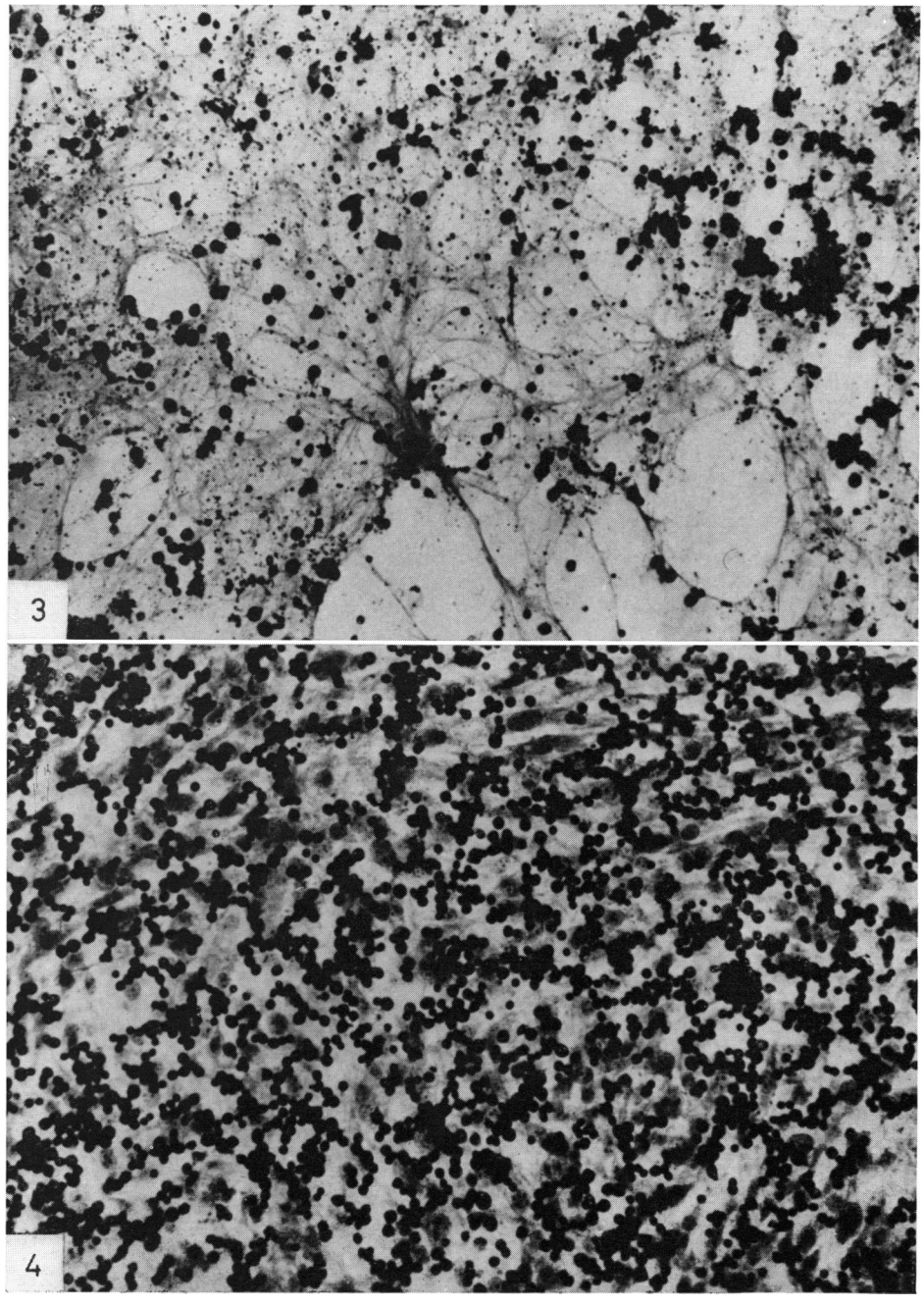

FIG. 3. An area from a plate seen in Fig. 1 of a transfer from C3H/J to C3H/J. The monolayer was lysed completely and only fibrous film left. A rare fibroblast surviving can be seen (low centre). Lymphoid cells are seen as dark round bodies. May–Grünwald–Giemsa, × 160.

FIG. 4. An area from a plate seen in Fig. 1 of a transfer from Wistar to C3H/J. No lysis of fibroblasts. Many lymphoid cells adhered to the monolayer. May–Grünwald–Giemsa, × 160.

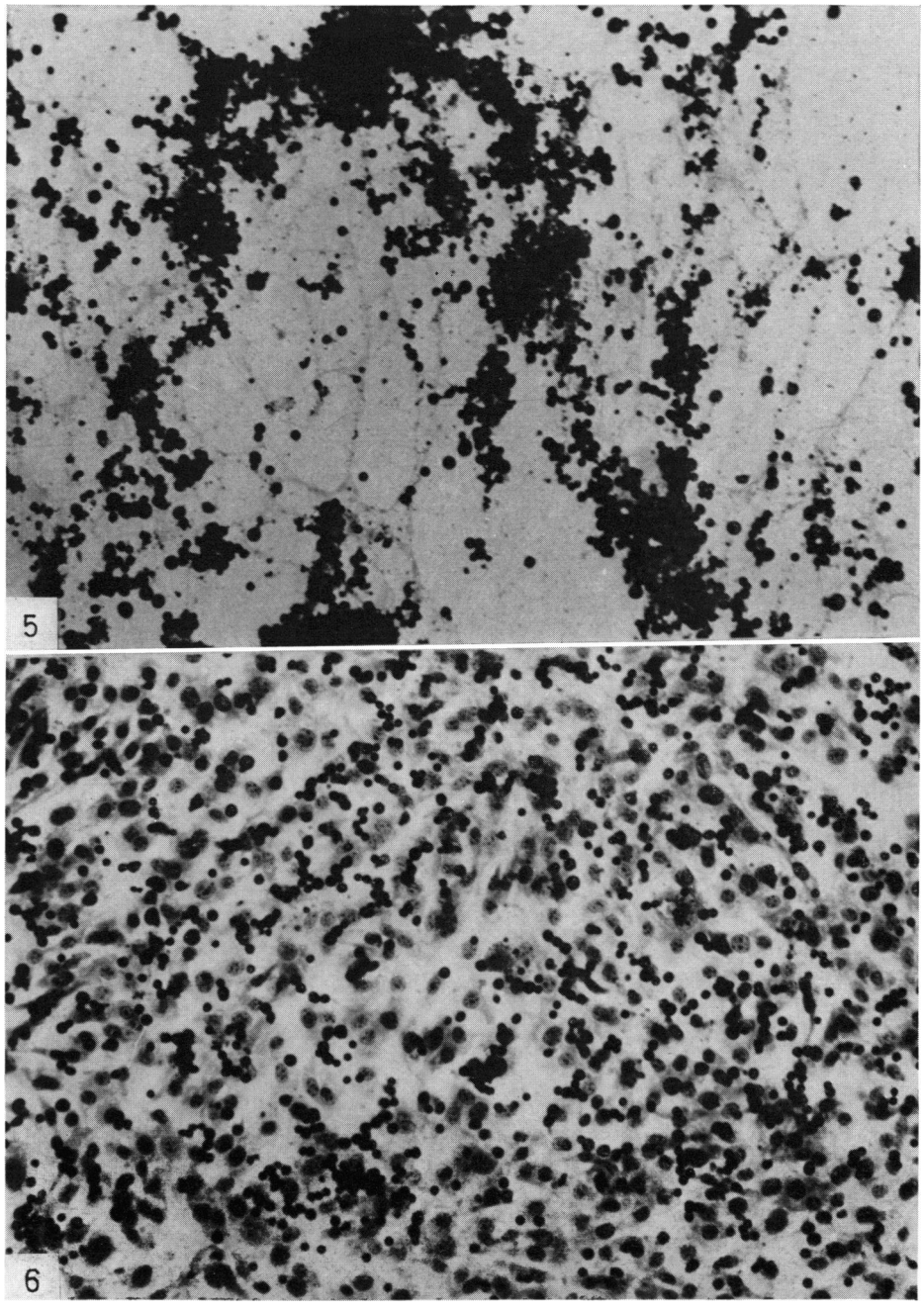

FIG. 5. An area from a plate seen in Fig. 1 of a transfer from C57BL/6 to C57BL/6. The monolayer was lysed overnight and only delicate fibrous mesh left. The lymphoid cells are seen arranged in clumps. May–Grünwald–Giemsa, × 160.

FIG. 6. An area from a plate seen in Fig. 1 of a transfer from C3H to C57BL/6. The monolayer is unaffected. Only slight lysis occurred. May–Grünwald–Giemsa, × 160.

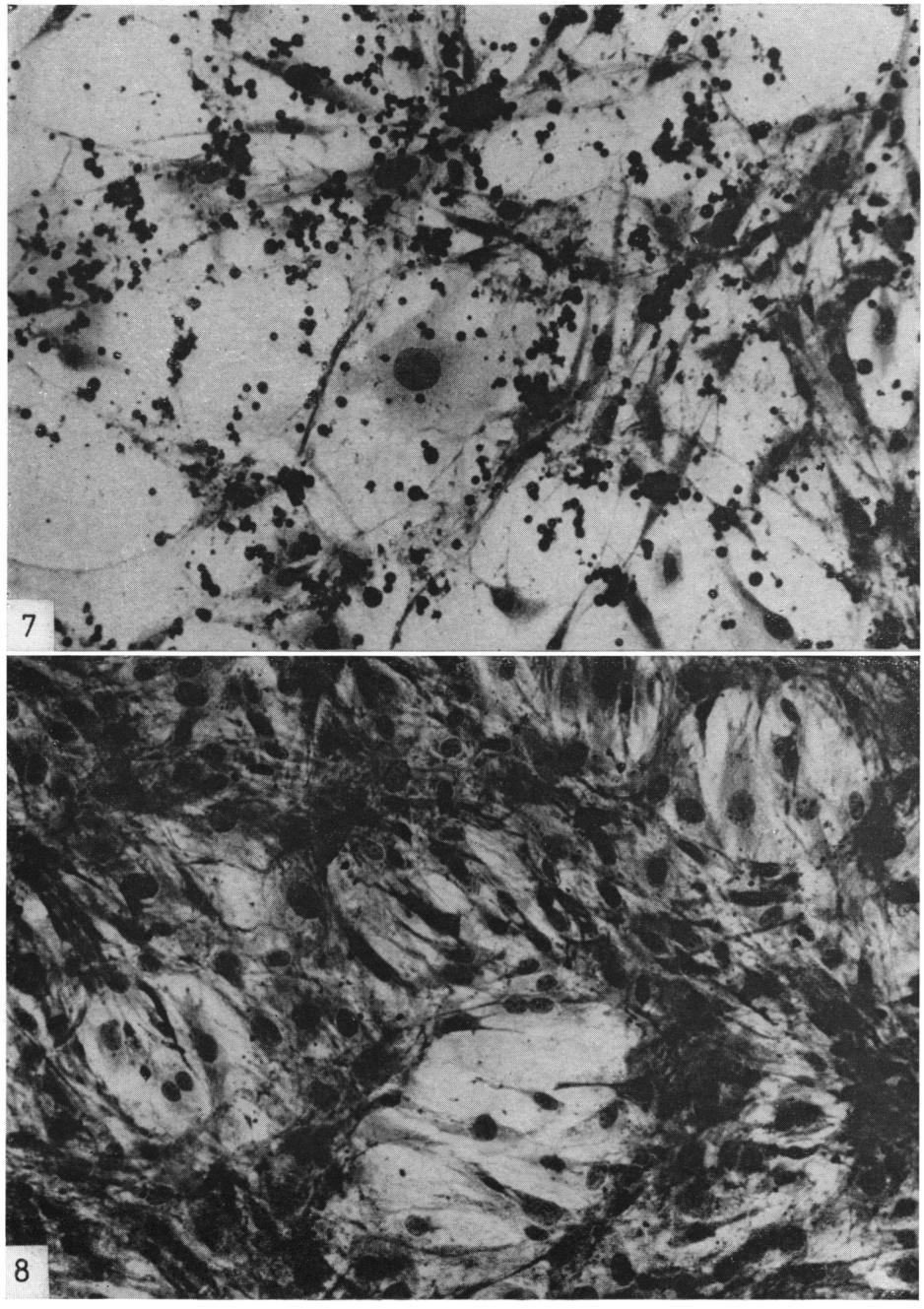

FIG. 7. An area of lysed monolayer from a plate seen in Fig. 1 of a transfer from BALB/C to BALB/C. The monolayer was lysed severely and only few fibroblasts survived. May–Grünwald–Giemsa, × 160.

FIG. 8. An area of a control monolayer of BALB/C from a plate seen in Fig. 1. May–Grünwald–Giemsa, × 160.

that are determined by the H-2 locus of the mouse. It strongly suggests that a given population of LPC originated on a monolayer of any strain is not necessarily sensitized only to one antigen, and this provides a possible explanation of differences in the rate of lysis obtained upon transfers to monolayers of other strains.

GRAFT REACTION CULTURES AMONG STRAINS OF RATS

The few studies carried out on lysis by LPC grown on allogeneic monolayers (Ginsburg, 1965) have indicated that lysis occurs, though not as strongly as in the case of mouse monolayers. Up to now, lysis has been demonstrated by lymphoid cells from Lewis strain versus BN (Table 2) and by lymphoid cells from Sprague–Dawley *versus* BN and Wistar. Many typical LPC cultures had either no lytic effect or only a very weak one. In general, rat lymphoid cells grown on rat monolayers formed a richer and healthier lymphoid population than those grown on mouse monolayers. LPC originated on a rat monolayer (Table 5 and Figs. 1 and 4) did not significantly affect mouse monolayers, although a few fibroblasts were observed undergoing lysis in such cultures. The difference in number of fibroblasts observed in monolayers seeded with LPC originated on Wistar from those of the control monolayers (Table 5) may be due either to this sporadic lysis, ascribable to some allotypic sharing of rat and mouse, or to a slower rate of division of the fibroblasts.

INOCULATION OF MOUSE MONOLAYERS WITH HOMOGENATE OF RAT LPC

In an attempt to test whether the reaction can be transferred by subcellular fractions or whether it is mediated by intact cells only, cell-free preparations of LPC were employed. A suspension of LPC was obtained 3 days after transfer of rat LPC originated on BALB/C to BALB/C monolayer. At this time, the monolayer was lysed completely. The suspension, collected from six plates, contained $53{\cdot}5 \times 10^6$ large cells and 27×10^6 small cells. An aliquot equivalent to one donor plate ($8{\cdot}9 \times 10^6$ large cells and $4{\cdot}6 \times 10^6$ small cells) was re-suspended in fresh medium and plated on originator monolayer. The monolayer was lysed completely within 19 hours. The rest of the cell suspension (44×10^6 large cells and 23×10^6 small cells) was centrifuged at 1000 rev/min (International Clinical Centrifuge, Head rotor No. 215) for 10 minutes, the cells were re-suspended in 1 ml PBS, and the suspension was then frozen at $-20°$. On the following day, the cells were thawed, homogenized and centrifuged at 2500 rev/min for 10 minutes. The supernatant was diluted 1:1 with fresh medium and plated on originator monolayer. The pellet was resuspended in 2·5 ml of fresh medium and plated on an originator monolayer. Observation over 6 days revealed no effect.

DISCUSSION

The data derived from the present series of experiments show:

(1) LPC originated on mouse strain monolayers consistently lysed other monolayers of the same batch or strain to a greater extent, or faster, than monolayers of other strains.

(2) When two mouse strains did not differ in the H-2 locus, for example BALB/C and DBA/2 (both H-2^d), LPC originated on either strain lysed monolayer of the other to the same extent and as quickly as that of the originator.

(3) Great variability was manifested by LPC originated on a monolayer of a particular

mouse strain as regards its ability to lyse monolayers of other strains that differed in their H-2 locus. It was also demonstrated that LPC originated on rat monolayers were unable to lyse mouse monolayers and vice versa.

A possible explanation for differences in lytic behaviour might be differences in the quality of the monolayers as supporters of lymphoid populations. If a monolayer maintains the suspension poorly, the LPC might fail to survive or lose their functional ability. The present results do not support such a contention—the same monolayer which might be a poor supporter of cells originated on one monolayer supported cells originated on others. In cross-transfers, no direct correlation was found between absolute numbers of active LPC and lytic potency. It may be postulated that some monolayers display refractoriness to lysis by LPC. If such were the case, the monolayer would neither be lysed by LPC originated on a monolayer of the same batch or strain nor by LPC from any other strain. It appears that each population of LPC originating on any batch of a particular strain of cells, acquired its own spectrum of specificity *in vitro* to lyse cells of strains of mouse or rat. This spectrum is a result of sensitization of a proportion of small lymphocytes to one or more of the tissue antigens of the strain complement present in the cell monolayer.

Consideration of LPC transfers between monolayers of strains that differ in the H-2 locus and those that do not, suggests that the tissue antigens involved are predominantly those determined by the H-2 locus of the mouse. A definite pattern can be observed in the extent or rate of lysis caused by LPC originated on a particular strain in other strains. This is most evident in the cross-transfers of LPC among BALB/C–C57BL/6–C3H strains. LPC which originated on any one of these three strains never completely lysed both of the others. This pattern suggests the involvement of the H-2 allelic complement, as these three strains do not share any common allele.

Since we are dealing with a strong xenogeneic barrier, it might be expected that those tissue antigens that specify the species differences will be more strongly antigenic to rat than those that specify the strain histocompatibility differences. Thus, there should be sensitization and lysis of all mouse monolayers, irrespective of the strain. The evidence suggests that this is not necessarily so. Mouse cells possess many antigens that can cause an immune reaction in the rat. Such antigens will elicit circulating antibodies. Even immunization across the H-2 histocompatibility barrier of the mouse elicits the production of isoantibodies which have been shown not to be involved in the mechanism of graft rejection (Sachs and Feldman, 1958). Evidence derived from the present study and from additional unpublished studies have suggested that this *in vitro* lysis is mediated by living LPC and not by a detectable extracellular agent.

It is suggested that this lysis, as was shown by Brent, Brown and Medawar (1962) for graft rejection, is an immune reaction of the delayed hypersensitivity type, and as these authors have shown, it is also a specific reaction. Other types of immune reaction which may be elicited *in vivo* were not obtained in this tissue culture sustem. On *in vivo* inoculation of mouse cells into a rat, the H-2 antigens may elicit a hypersensitivity reaction which underlies the mechanism of graft rejection while these or other antigens of the mouse may also elicit the production of circulating antibodies. We believe that in our tissue culture system those configurations which specify the H-2 antigenic differences were involved in the sensitization, or, at least, were the only ones that stimulated the lytic reaction.

Considering the identity in development and in morphology of the cultures of LPC and the known *in vivo* graft versus host reaction found when rat lymphocytes are inoculated into the mouse (Gesner and Gowans, 1962), allied to the fact that both reactions cause severe tissue damage, one is led to the conclusion that the *in vitro* reactions described in the present study represent a graft *versus* host reaction, where the embryo monolayer constitutes the recipient host of the inoculated lymphocytes.

ACKNOWLEDGMENTS

This research was supported in part by grants from the United States Public Health Service AM-04826-05, 5 RO1 HE 03174 and C-6165 and by the American Cancer Society Institutional Research Grant No. 5890. The author wishes to express his gratitude to Dr E. P. Benditt and Dr M. Feldman for their encouragement through all the phases of the work, and to Dr G. Martin and Dr D. Lagunoff for their kind support and helpful discussions. The skilful technical assistance of Mrs C. Potter Norman and Miss M. Debby and the help of Mrs M. Derr are greatly appreciated.

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