

## SPECIFIC ADHERENCE OF IN VITRO DIFFERENTIATED LYMPHOCYTES TO TARGET CELLS\*

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The involvement of cellular immunity in transplantation rejection is considered to take part in two stages: (a) the sensitization stage of the small lymphocyte, and (b) the effector stage of target-cell destruction. In addition, grafting a transplant leads to development of a hypersensitive state to the transplantation antigens. Both stages involve a contact between lymphocytes or the effector cells on one hand and the incompatible target cells on the other hand (1-5). In the sensitization stage a small fraction of small lymphocytes that presumably bear suitable receptor for the transplantation antigen undergo activation (6-8). This activation culminates in transformation of the lymphocytes to blast cells and in the appearance of lymphocytes hypersensitive to the specific transplantation antigen. Both blast cells and hypersensitive lymphocytes are considered to be the effector cells in transplantation rejection. Since contact is needed in these processes, it is reasonable to think that the immunological specificity of cellular immunity lies in the contact between the cells. The ability to study, experimentally, the nature of this contact would be of great value in understanding the immunological basis of cell recognition and cell destruction. However, this goal requires a clean population of lymphocytes that have a receptor for a certain specificity.

In recent publications (9, 10) we have shown that blast cells, obtained after stimulation of rat lymphocytes with pokeweed mitogen (PWM),<sup>1</sup> lyse monolayers composed of embryo fibroblasts when PWM is present. This target-cell lysis is similar to that obtained after stimulation of rat lymphocytes with xenogeneic (mouse) fibroblast monolayers (11). It is important to mention here that preincubation of syngeneic fibroblast monolayers with PWM and washing away the excess of PWM before the addition of the lymphoid cell produced the same effects; namely, PWM combined with the fibroblasts and the resulting "conjugate" (designated as fibroblast-PWM-cells) was lysed by the PWM-blast cells (9). This in vitro system is shown schematically in Fig. 1.

The behavior of PWM-blast cells on the fibroblast monolayers in the absence of PWM was of particular interest. The blast cells could not lyse target cells; instead,

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<sup>1</sup> Abbreviations used in this paper: Con A, concanavalin A; LA, 5% lactalbumin hydrolysate in Earle's solution + 5% calf serum; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; PWM, pokeweed mitogen.

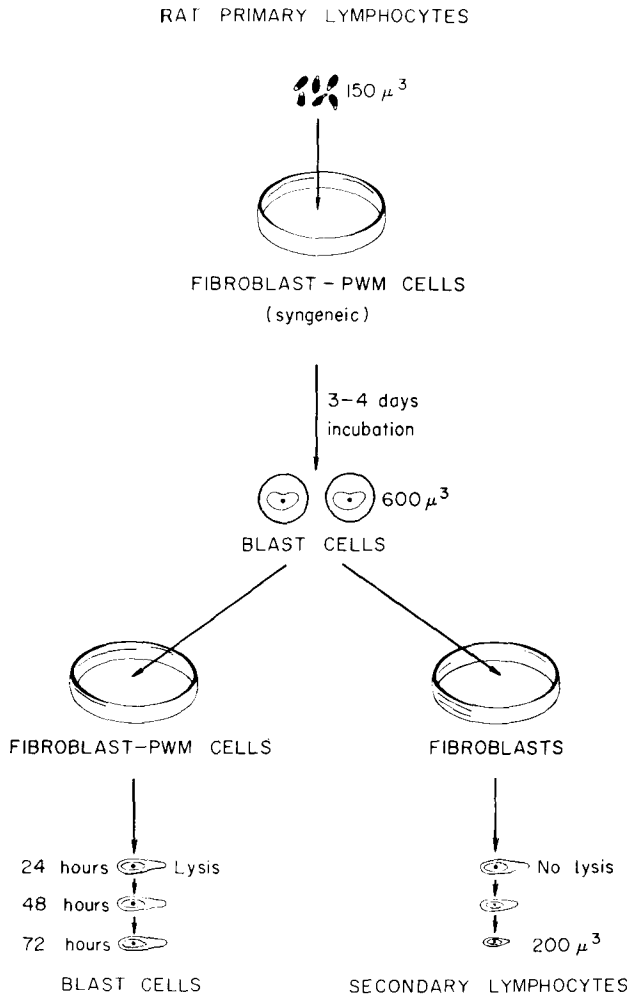


FIG. 1. Schematic representation of the culture system employing fibroblast-PWM cells for obtaining blast cells and PWM-lymphocytes from rat primary lymphocytes.

they transformed within 2-3 days into a distinctive type of lymphocyte termed "secondary lymphocyte." (We termed the ordinary, small lymphocyte taken directly from the animal as "primary lymphocyte.") Blast cells obtained after stimulation of primary lymphocytes with phytohemagglutinin (PHA) and concanavalin A (Con A) also transformed into secondary lymphocytes when grown on monolayers in the absence of a mitogen. We have thus obtained populations of three different classes of secondary lymphocytes (termed PWM-lymphocytes, PHA-lymphocytes, and Con A-lymphocytes). By testing the lytic ability of the three classes of secondary lymphocytes, we have found that only PWM displays an immunological specificity. PWM-lymphocytes lyse target cells with considerably more efficiency in the presence of

PWM than do PHA-lymphocytes or Con A-lymphocytes. However, no specificity was demonstrated when the lysis was tested in the presence of either PHA or Con A (10).

In our study with the secondary lymphocytes we were struck by an interesting phenomenon: when we added PWM to the cultures, the suspension of PWM-lymphocytes disappeared within a short time and all the cells were seen firmly attached to the fibroblasts. Suspensions of Con A-lymphocytes and PHA-lymphocytes, on the other hand, remained intact. The present paper reports the quantitation and kinetics of this particular aspect of cellular immunity, namely, the recognition of target cells as manifested by specific cell contact.

### *Materials and Methods*

*Animals.*—Lymphocytes and embryos for preparation of monolayers were obtained from rats of the Lewis inbred strain.

*Mitogens.*—A stock solution of pokeweed mitogen (Grand Island Biological Co., Grand Island, N.Y.) containing 1 mg dry weight/ml distilled water was made up and kept frozen. A stock solution of PHA (Difco Laboratories, Detroit, Mich.) containing 20 mg dry weight/ml distilled water was kept at 4°C. Con A (Miles-Yeda Ltd., Rehovot) was dissolved in a saturated solution of NaCl, and a stock solution containing 25 mg/ml was kept at room temperature.

*Embryo Fibroblast Monolayers.*—Monolayers from embryos were prepared and maintained as previously described (12, 13). Monolayers used for maintenance of growth and differentiation of blast cells and secondary lymphocytes were prepared by plating  $5 \times 10^6$  fibroblasts in 10 ml of LA medium (5% lactalbumin hydrolysate in Earle's solution + 5% calf serum) onto 100-mm Falcon plastic Petri dishes (Falcon Plastics, Div. B-D Laboratories, Inc., Los Angeles, Calif.) 3–6 days later lymphoid cells were plated on the monolayers. Monolayers used as targets for adherence of lymphocytes were prepared by plating  $1 \times 10^6$  to  $2 \times 10^6$  fibroblasts in 4 ml of LA medium onto 60-mm plastic Petri dishes. 1–2 days later the lymphocytes were plated onto the monolayers.

*Stimulation of Primary Lymphocytes with Mitogens.*—Suspensions of lymphocytes were prepared from lymph nodes as described previously (14). Lymph nodes were placed in phosphate-buffered saline (PBS) and forced through a wire mesh using a syringe plunger. The resulting suspensions were resuspended in Dulbecco's medium containing 15% horse serum. Mitogens were added to the suspensions to give the following concentrations: PWM, 0.005 ml of stock solution per ml of medium (5  $\mu\text{g}/\text{ml}$ ); PHA, 0.003 ml of stock solution per ml of medium (60  $\mu\text{g}/\text{ml}$ ); Con A, 0.001 ml of stock solution per ml of medium (25  $\mu\text{g}/\text{ml}$ ).  $3 \times 10^7$  cells in 4 ml were then plated onto 60-mm Falcon Petri dishes and the cultures were incubated at 37°C in a humidified incubator with a flow of 7%  $\text{CO}_2$  in air. Cultures with PHA or Con A were incubated for 3 days, while those with PWM were incubated for 4 days.

*Growth of Blast Cells on Embryo Monolayers.*—Blast cells were grown on embryo monolayers as previously described (10). After 3–4 days of incubation with a mitogen the cultures were very rich in blast cells. The cells were harvested, washed once with medium, and resuspended in a fresh mitogen-free medium. 10 ml of the cell suspension were plated onto a rat embryo monolayer in a 100 mm Petri dish, at a concentration of  $2 \times 10^6$  cells per dish. In order to obtain suspensions of secondary lymphocytes the cultures were further incubated for 3 days.

*Adherence of Lymphocytes to Target Cells.*—The degree of adherence was assayed in two ways: (a) by labeling the lymphocytes with  $^{51}\text{Cr}$ , and (b) by counting the cells in a hemacytom-

eter. In assay (a) lymphocyte suspensions containing  $10^7$  cells/ml were incubated with 40  $\mu$ Ci of sodium chromate- $^{51}\text{Cr}$  (The Radiochemical Centre, Amersham, England) for 1 hr at 37°C. The suspensions were then washed three times. In both assays (a) and (b)  $3 \times 10^6$  secondary lymphocytes (either labeled or unlabeled) in 4 ml of medium were plated onto a fibroblast monolayer in 60-mm Petri dishes. 24 hr later 0.5 ml of mitogen solution in medium was added to each culture. The final concentration in culture for each mitogen was as follows: PWM, 20  $\mu\text{g}/\text{ml}$ ; Con A, 50  $\mu\text{g}/\text{ml}$ ; and PHA, 100  $\mu\text{g}/\text{ml}$ . At intervals the free-floating lymphocytes were collected by pipetting of the culture and the monolayer was washed once with additional 4 ml of medium which were combined with the first harvest. This represents the medium fraction. The remaining monolayer was overlaid with trypsin solution containing 0.005% neutral red and the plates were incubated for 30 min. After it was shaken, the trypsinized suspension was collected into a tube. This represents the fibroblasts fraction. When labeled lymphocytes were assayed, the medium fraction was centrifuged, the supernatant fluid was discarded, and the cells in the pellet were resuspended in 3 ml of medium. Each fraction was then counted in a well-type Packard Autogamma spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). The total activity in a plate is given by the radioactivity obtained in the fibroblast fraction plus the radioactivity in the medium fraction. Adherence is expressed as the per cent of the total radioactivity found in the fibroblast fraction. In assay (b) mean value was obtained from eight hemacytometer counts of each sample. Neutral red stains the fibroblast and the lymphoid cells quite differently (11). Adherence is expressed as per cent of the total number of lymphoid cells obtained in the fibroblast fraction. The difference in replicate plates did not exceed 10% and the standard deviation was therefore omitted from tables.

*Target-Cell Lysis.*—The degree of target-cell lysis was assayed by the release of  $^{51}\text{Cr}$  as previously described (13). Briefly,  $0.8 \times 10^6$  Lewis fibroblasts in 1.5 ml of medium were plated in 35-mm Falcon plastic Petri dishes. 24 hr later the cultures were X-irradiated with 2000 R. The medium was then replaced with 1 ml of medium containing 1.5  $\mu$ Ci of sodium chromate- $^{51}\text{Cr}$ . After 24 hr the medium was again replaced with 1.5 ml of fresh medium and the plates were incubated for an additional 2–4 days. In order to assay the release of  $^{51}\text{Cr}$ , suspensions of lymphoid cells in 1.5 ml of Dulbecco's medium with 15% horse serum were plated onto the  $^{51}\text{Cr}$ -labeled monolayers. The cultures were incubated at 37°C for 22 hr and processed as previously described (13). The medium from each plate was collected and 1.5 ml of PBS was added to the monolayer. The plates were then shaken and the PBS was collected and combined with the medium. This represents the "medium fraction." The monolayers remaining in the plates were overlaid with 1.5 ml of trypsin solution and the plates were incubated for 20 min. After it was shaken, the trypsinized suspension was removed, 1.5 ml of distilled water was added to the plates, and 30 min later the supernatant was combined with the trypsin suspension. This fraction represents the "fibroblast fraction." Each fraction, containing 3 ml of fluid, was counted in a well-type iodide sodium Crystal Packard Autogamma spectrometer. The total radioactivity in a plate is given by the radioactivity obtained in the fibroblast fraction plus the radioactivity in the medium fraction. Lysis is expressed as the per cent of total radioactivity released into the medium after correction for the release of  $^{51}\text{Cr}$  in control plates:

$$\frac{{}^{51}\text{Cr in presence of mitogen} - {}^{51}\text{Cr release in absence of mitogen}}{\text{Total radioactivity} - {}^{51}\text{Cr release in absence of mitogen}} \times 100.$$

## RESULTS

*Stimulation of Primary Lymphocytes with Fibroblast-PWM Cells.*—In our previous work (10) we produced blast cells by exposing a highly concentrated lymphocyte suspension ( $30 \times 10^6$  lymphocytes in 60-mm Petri dishes) to PWM in the absence of a fibroblast monolayer. The situation is very different when

lymphocytes are stimulated in the presence of a fibroblast monolayer. Excellent populations of blast cells are obtained; however, one must plate a considerably smaller number of lymphocytes. Stimulation of more than  $10^6$  to  $2 \times 10^6$  lymphocytes leads to a rapid and severe drop in pH accompanied by the complete death of the culture (10). Table I represents a typical experiment.  $2 \times 10^6$

TABLE I  
*Stimulation of Rat Lymphocytes by Syngeneic Fibroblast-PWM Cells\**

Culture of lymphocytes on fibroblast monolayer	Blast cells harvested	Lysis of target cells†	
		No. of lymphoid cells plated ( $\times 10^6$ )	$^{51}\text{Cr}$ release
	%		%
Fibroblast-PWM cells			
Adhering lymphocytes§	64.0	1.00	45.9
		0.12	28.1
Nonadhering lymphocytes	7.9	1.00	4.2
		0.12	3.3
Unseparated lymphocytes¶	61.0	1.00	42.1
		0.12	27.9
Fibroblasts			
Unseparated lymphocytes**	0.0	1.00	7.2
		0.12	3.1

\*  $2 \times 10^6$  Lewis embryo fibroblast cells were plated onto 60-mm Petri dishes. 24 hr later the plates were irradiated with 2000 R X-rays and incubated for 24 hr with 4 ml of medium containing 40  $\mu\text{g}$  PWM/ml. The plates were washed twice with medium before Lewis lymphocytes were added.

† Titration of target-cell lysis was performed in the presence of 10  $\mu\text{g}$  PWM/ml. The plates were incubated for 22 hr.

§  $7 \times 10^6$  lymphocytes were plated and incubated for 10 hr. The nonadhering cells were removed and fresh medium was added. The cultures were further incubated for 62 hr.

||  $7 \times 10^6$  lymphocytes were plated and incubated for 10 hr. The nonadhering cells were collected and replated on fibroblast-PWM cells at a concentration of  $1.5 \times 10^6$  lymphocytes per plate. The cultures were further incubated for 62 hr.

¶  $1.5 \times 10^6$  lymphocytes were plated onto each plate. The cultures were further incubated for 72 hr.

\*\* Same as ¶ but  $7 \times 10^6$  lymphocytes were plated.

Lewis embryo fibroblast cells were incubated for 24 hr with medium containing 40  $\mu\text{g}$  PWM/ml. These fibroblasts (termed fibroblast-PWM cells) were washed twice and then either  $1.5 \times 10^6$  or  $7 \times 10^6$  Lewis lymphocytes were added. The more concentrated culture was incubated for 10 hr, after which the nonadhering lymphocytes were harvested by pipetting and replated at a concentration of  $1.5 \times 10^6$  cells onto a new fibroblast-PWM cell monolayer. Fresh medium was then added to the donor culture. Since about 10-20% of primary lymphocytes adhere to fibroblasts in the presence of PWM (*vide infra*), also here about  $1.5 \times 10^6$  lymphocytes were left after the nonadhering cells were removed. The three

groups of cultures (the unseparated lymphocytes, the nonadhering lymphocytes, and the adhering lymphocytes) all now at a concentration of  $1.5 \times 10^6$  per plate, were incubated for a further 62 hr. The blast cells were then harvested and assayed for lysis of target cells by the  $^{51}\text{Cr}$  assay method (13).

As seen in Table I the nonadhering lymphocytes did not lyse the monolayer, whereas significant lysis was obtained by the adhering as well as the unseparated lymphocytes. These two cultures lyse the monolayer to about the same extent, indicating that the lytic potential was located wholly in the adhering portion of the lymphocyte population. No lymphocyte transformation occurred in the untreated, PWM-free monolayer. This suspension was composed purely of lymphocytes during 72 hr of incubation. In contrast, a high proportion of blast cells was obtained at this time in the culture of the adhering lymphocytes.

*The Adherence of Primary Lymphocytes to the Fibroblast Monolayer.*—Lymphocyte suspensions prepared from lymph nodes of normal rats were assayed for adherence to fibroblast monolayers in the presence of mitogens by the direct lymphocyte count method. Mitogens were added 24 hr after the lymphocytes were plated on the monolayer. Table II shows the rate of adherence induced by

TABLE II  
*Adherence of Rat Primary Lymphocytes to Syngeneic Fibroblasts After Addition of Mitogens*

Time after addition of mitogen	Mitogen			
	PWM	Con A	PHA	None
<i>hr</i>		<i>% adherence</i>		
3	11.5	34.7	67.6	6.9
6	19.9	61.3	45.6	6.6

the three mitogens. Con A and PHA induced high rates of adherence. In contrast, PWM induced only slight adherence; within 6 hr the adherence was 13% more in the presence of PWM than in control cultures. The result with PHA was rather surprising. This mitogen constantly showed high agglutinating ability; however, in many instances part of the lymphocytes detached later.

The kinetics of the adherence during 34 hr of incubation is shown in Table III. It was performed by using fibroblast-PWM cells rather than by adding PWM directly to the cultures. During this period only a small number of lymphocytes adhered in the presence of PWM. The first sign of transformation of adherent lymphocytes was seen as early as 20 hr and at 34 hr typical blast cells were identified.

*The Adherence of Secondary Lymphocytes.*—The adherence of PWM-lymphocytes, Con A-lymphocytes, and PHA-lymphocytes was assessed after the three mitogens were added to the cultures. Five experiments performed by direct lymphocyte count produced identical results. A representative experiment is shown in Table IV. PWM induced a very high proportion of PWM-lympho-

TABLE III  
*Adherence of Primary Lymphocytes to PWM-Treated Fibroblasts*

Time	Adherence of lymphocytes (per cent)	
	Fibroblast-PWM cells*	Fibroblasts
<i>hr</i>		%
3	11.7	10.1
6	10.8	8.1
10	10.6	8.7
20	17.3	9.7
34	18.3	14.5

\* Rat Lewis embryo fibroblasts syngeneic to the lymphocytes were incubated for 24 hr with medium containing 40  $\mu$ g PWM/ml. After the monolayer was washed twice, lymphocytes were plated.

TABLE IV  
*Adherence of Secondary Lymphocytes to Syngeneic Fibroblast Monolayers After Addition of Mitogens*

Time after addition of mitogen	Class of lymphocytes	Mitogen			
		PWM	Con A	PHA	None
<i>hr</i>		% adherence			
3	PWM-lymphocytes	75.1	78.0	54.6	10.5
	Con A-lymphocytes	28.0	63.0	67.2	5.3
	PHA-lymphocytes	16.8	73.7	73.4	5.1
6	PWM-lymphocytes	81.4	93.2	67.6	12.5
	Con A-lymphocytes	45.0	93.0	73.8	7.2
	PHA-lymphocytes	25.3	89.3	79.4	8.3

cytes to adhere during 3–6 hr. PWM also induced a significant number of Con A-lymphocytes but only a small number of PHA lymphocytes. The PWM-stimulated levels of adherence shown in Table IV for the three classes of secondary lymphocytes were reproduced in all our experiments indicating clear differences among the lymphocytes in affinity to PWM.

Entirely different results were obtained when Con A and PHA were added to the cultures. Both mitogens induced high rate of adherence of the three classes of lymphocytes without showing clear specificity. The kinetics of PWM-stimulated adherence of secondary lymphocytes was determined by using  $^{51}\text{Cr}$ -labeled cells. In Fig. 2 adherence of PWM-lymphocytes is seen as early as 20 min after the addition of PWM, and a plateau of maximal adherence of 75–80% is reached between 2 and 3 hr. Preliminary studies indicated that the use of  $^{51}\text{Cr}$  is less accurate than direct lymphocyte count in assessing the level of lymphocyte adherence. The reason for this is the high background values (35–40% between 2 and 3 hr) obtained in the control cultures in the absence of mitogen. We have

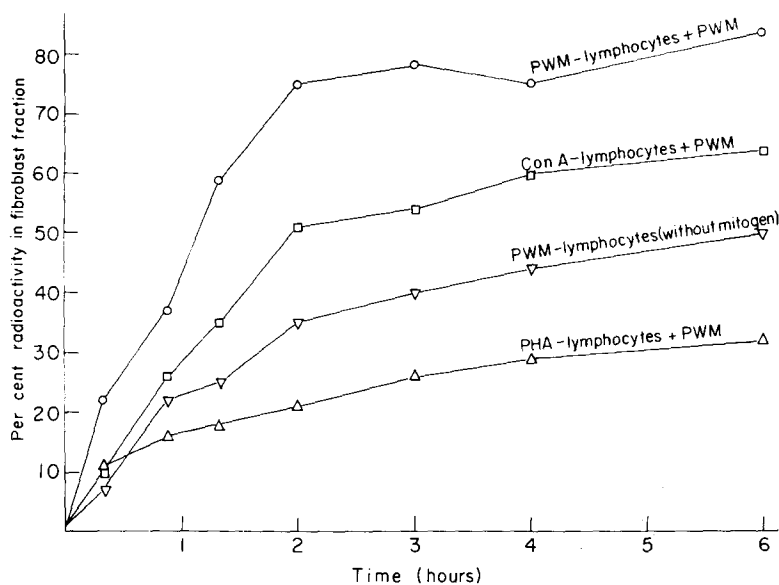


FIG. 2. Mitogen-induced adherence of secondary lymphocytes as a function of time.

confirmed that the  $^{51}\text{Cr}$  is not reutilized by the fibroblasts (15). Thus, it seems possible that the  $^{51}\text{Cr}$ -labeled lymphocytes might tend to adhere in a more non-specific manner than the unlabeled lymphocytes. Despite this limitation, the  $^{51}\text{Cr}$  technique was found useful for studies where direct lymphocyte count proved to be impractical.

*The Effect of Temperature on the Capacity of Adherence.*—In addition to the usual incubation at  $37^\circ\text{C}$ , the primary lymphocytes and PWM-secondary lymphocytes shown in Tables II and IV were incubated at  $22^\circ\text{C}$  and the results obtained are shown in Table V. The adherence induced by PWM and by Con A was much slower at  $22^\circ\text{C}$  and fewer lymphocytes adhered. On the other hand, the low temperature did not affect the adherence induced by PHA. It should be noted that there was higher percentage of nonspecific adherence at  $22^\circ\text{C}$  than the adherence at  $37^\circ\text{C}$  in the control cultures without mitogen.

#### DISCUSSION

It has been shown by several workers that in the graft reaction both the recognition phase and the effector phase involve contact with the target cells. In the recognition phase adherence of small lymphocytes to target cells is a prerequisite (1). The adherent lymphocytes are activated and transform to blast cells. In the effector phase both the blast cells and the hypersensitive lymphocytes participate in target-cell lysis. Such cells can be obtained also from immune animals and exposed in vitro to the specific target cells. Already in 1961 Rosenau and Moon (16) claimed that target-cell lysis involves contact between



TABLE V  
*Adherence of Lymphocytes to Syngeneic Fibroblasts at 22°C*

Class of lymphocytes	Time after addition of mitogen <i>hr</i>	Mitogen			
		PWM	Con A	PHA	None
PWM-lymphocytes*	3	28.7	46.0	66.0	23.9
	6	42.0	48.0	58.1	23.8
Primary lymphocytes‡	3	11.2	12.8	74.0	6.0
	6	17.2	22.4	75.0	15.9

\* Compare with Table IV which shows the results obtained at 37°C.

‡ Compare with Table II which shows the results obtained at 37°C.

the immune lymphocytes and the target cells. This has been demonstrated also by others (2-5). We have studied the contact of blast cells to target cells with time-lapse cinematography (17). The blast cells achieve contact by means of a tail-like protrusion, the "uropod" described by McFarland, Heilman, and Moorhead (18).

The present paper further substantiates the notion that cellular immunity functions through contact of the lymphoid cells with the immunologically incompatible cells. In this study we have used plant lectins which become bound to the cells. PWM is of particular interest since it stimulates a specific cell-mediated immune response (10). Here we show that this specificity is manifested in the adherence to target cells. In the case of primary lymphocytes a small fraction of the lymphocytes adhere in the presence of PWM. There is a straight correlation between the rate of adherence and the rate of transformation: only a fraction of the lymphocytes are transformed by PWM (10). In contrast, Con A and PHA apparently induce a total transformation with a directly correlated degree of adherence of lymphocytes. Indeed, the effect of PWM on the rate of adherence and transformation is more similar to the effect obtained by xenogeneic mouse fibroblast monolayer (11). These, among other things, led us to the belief that PWM, once bound to the cell membrane, acts as a transplantation antigen (10).

Unlike primary lymphocytes, PWM-secondary lymphocytes totally adhere in the presence of PWM. It thus appears that all the cells in the population of PWM-lymphocytes bear a receptor specific to PWM. In the population of primary lymphocytes only a fraction of the cells carry a receptor to the PWM. Since the PWM-lymphocytes developed from primary lymphocytes through blastogenesis (mitosis and transformation of the blast cells), it appears that the specific configuration on the cell membrane has been synthesized during the development of the hypersensitive lymphocytes.

Once the PWM-stimulated primary and secondary lymphocytes have adhered to target cells, they undergo an activation which culminates in their trans-

formation into blast cells. Yet, there are certain differences between the primary and secondary lymphocytes in this context as well. We have been carefully watching the transformation course. In cultures with primary lymphocytes the first signs of cell enlargement have been seen as early as 20 hr after the addition of PWM, and at 34 hr clear blast cells can be identified. In the cultures with PWM-lymphocytes cell enlargement was seen as early as 6 hr after the addition of PWM, and the blast cells were evident at 16 hr. In these cultures the entire population transformed. A comparative study of the transformation of both primary and secondary lymphocytes is now in preparation.

The behavior of Con A-lymphocytes and PHA-lymphocytes in the presence of PWM merits a comment. A constant ratio was observed among the adhering cells of the three classes of lymphocytes: PWM-lymphocytes were always highest, Con A-lymphocytes in between, and PHA-lymphocytes the lowest. This relationship to PWM suggests that each of the secondary lymphocytes has its own distinct properties. Since Con A and PHA induce extensive adherence and transformation of all types of lymphocytes, no clear specificity is demonstrated in the part these mitogens play. However, the lymphocytes derived from these mitogens might have acquired specific membrane configurations. This specific potential may be expressed in their response to other stimulants, e.g., to PWM. Based on the present results consideration should be given to the possibility that specificity in target-cell recognition in this *in vitro* system is merely a matter of quantitative differences in affinity to target cells. This affinity can be high or low, albeit specific.

The preliminary work carried out at 22°C has indicated that adherence of PWM-lymphocytes to fibroblasts induced by PWM and Con A was slowed down by lowering the temperature. On the other hand, the adherence induced by PHA was not affected. These results suggest that the cells are not passively agglutinated by PWM and Con A; rather the adherence in these cases appears to be an active process performed by the motile lymphocytes and requiring metabolic activity.

#### SUMMARY

Blast cells which were derived from rat lymphocytes by stimulation with phytohemagglutinin (PHA), concanavalin A (Con A), or pokeweed mitogen (PWM) transformed within 2-3 days into a new type of lymphocytes when plated without mitogen on embryo fibroblast monolayers. These lymphocytes were termed secondary lymphocytes. Upon addition of PWM to PWM-secondary lymphocytes a marked adherence to fibroblast monolayers was observed. The degree of adherence was estimated (*a*) by direct count of the lymphocytes in the medium and in the trypsinized fibroblast fraction, and (*b*) by using <sup>51</sup>Cr-labeled lymphocytes. The adherence process required incubation at 37°C. The process started immediately after the addition of PWM and reached a plateau at 6 hr. At this time more than 80% of the lymphocytes adhered. In the absence

of PWM only 12% of the lymphocytes were found in the fibroblast fraction. Unlike PWM-lymphocytes, Con A-lymphocytes, PHA-lymphocytes, and ordinary lymphocytes taken directly from the rat lymph nodes adhered only slightly more in the presence of PWM (10–20% adherence of ordinary lymphocytes) than in its absence (8% adherence). The adherence of the secondary lymphocytes and the ordinary lymphocytes was also studied in the presence of Con A and PHA. These mitogens induced high rate of adherence and they did not demonstrate specificity in their action. The adherence was accompanied by transformation of the lymphocytes to blast cells endowed with target-cell lytic ability. This transformation occurred mostly in the adhering fraction of the lymphocyte population. The results support the notion that target-cell recognition and destruction in cellular immunity involve contact between the cells.

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