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Identification of mouse H-2 antigens by mixed lymphocyte culture in the presence of PHA. II. Blastformation of mouse lymphocytes in culture according to the difference in H-2 antigens

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Identification of mouse H-2 antigens by mixed lymphocyte culture in the presence of PHA. II. Blastformation of mouse lymphocytes in culture according to the difference in H-2 antigens*

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Abstract

In the mixed tissue culture of mouse lymphocytes with addition of PHA the rate of the appearance of large and intermediate cells increases markedly, but which side of the two cell groups have reacted stronger remains obscure. In order to solve this problem, mixed cultures were conducted in such a way that only one cell group of the two would react. Namely, one cell group was exposed to C0 6).irradiation (Table 2) prior to the culture and cultured with another viable cell group (FJ test group, Table 2) to see the percentage of the appearance of large and intermediate cells. Simultaneously, the skin homograft from respective donor mouse was transplanted to each other and the survival days of each skin graft were compared. As a result it has been shown that the percentage of blastformation and the survival time of the skin transplant in each group prove to be in an inverse relation. The results of these mixed cultures indicate that the extent of blast. formation reflects significantly the difference in B-2 histocompatibility antigens.

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IDENTIFICATION OF MOUSE H-2 ANTIGENS BY MIXED LYMPHOCYTE CULTURE IN THE PRESENCE OF PHA

II. BLASTFORMATION OF MOUSE LYMPHOCYTES IN CULTURE ACCORDING TO THE DIFFERENCE IN H-2 ANTIGENS

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It has been demonstrated that, when mouse lymphocytes are cultured in the medium containing PHA, either in single or mixed culture with other cells, the rate of large and intermediate cell appearance is markedly enhanced. However, when these lymphocytes are cultured in various combinations with other cells in the absence of PHA, there can be observed hardly any significant difference in the rate of blastformation between various combinations, while there is a distinct difference in the presence of PHA. This fact suggests that the H-2 antigen histocompatibility difference is made more marked on addition of PHA. It remains, however, still obscure which side of the cell groups in the combination responds more markedly. In order to solve this question, a series of mixed cell cultures were conducted under such conditions that only one group of the cells in the two-group combination was made to react. Namely, one group of the lymphocytes (A or C3H cells) was previously irradiated with Co^{60} to inhibit mitosis, and then one irradiated group was cultured with the other non-irradiated group: either in the combination of irradiated A + C3H cells or irradiated C3H + non-irradiated A cells. Simultaneously, F_1 mouse lymphocytes were cultured with their parent lymphocytes (as the F_1 test); F_1 + A or F_1 + C3H. These mixed cultures were conducted in order to see what relation would blastformation show with the survival time of the skin homograft transplanted.

MATERIALS AND METHODS

The methods employed here are identical with those used in Part I.

Lymphocyte: Lymphocytes are isolated from cervical and axillary lymph nodes of A mice (H-2^a), C3H mice (H-2^k), and (A×C3H) F_1 mice, both from male and female adult mice, as in the previous experiment.

Irradiation of the cells with Co^{60}: The irradiation is done under the conditions of 10^4R of Co^{60}; 200 kv; 25 mA; 0.54μ+0.5 Al filter; focal distance 20 cm; time 22′ 22″. After confirming the death of the lymphocytes by supravital staining (1, 2), the mixed cell cultures are carried out.

Culture medium: The medium is composed of TC-199 + YLE + Hanks solution + bovine serum, 5 : 2 : 1 : 2 (v/v).

PHA: One vial of hemagglutinin M (Difco Laboratories, Detroit, Michigan, U. S. A.) is dissolved in 5 ml of the medium. Antibiotics used is penicillin (Takeda Pharmaceutical K. K.).

Skin transplantation: The skingraft in the direction of $F_1 \rightarrow A$, and $F_1 \rightarrow C3H$ in full thickness is transplanted on the back of the mice. The rejection of the skingraft is determined macroscopically, and each of these two groups is composed of 20 animals.

Culture methods: In both single and mixed cultures 100×10^4 lymphocytes are used each time. The static cultures are carried out at 37°C for 72 hours, and the specimens are taken at certain intervals, stained with May-Giemsa stain, and the cells are classified as large, intermediate and small lymphocytes (3, 4).

RESULTS

1. *The mixed culture of Co^{60}-irradiated lymphocytes and non-irradiated normal lymphocytes:*

In the combination of irradiated A cells + normal nonirradiated C3H cells without addition of PHA the rate of appearance of large and intermediate cells was 7.0%, while in the reverse combination of non-irradiated A cells + irradiated C3H cells, the rate proved to be 5.35%, showing hardly any significant difference between them. In the presence of PHA, however, the rate of appearance in the former combination was 27.95% and 18.1% in the latter, indicating a distinct difference ($p=0.05$) between the two groups.

2. *Mixed cultures of F_1 cell + parent cells:*

In the combination of $A+F_1$ cells without PHA the rate of large-intermediate appearance (blastformation) was 7.45% while it was 9.70% in the combination of $C3H+F_1$, revealing hardly any significant difference between the two combinations. However, on the addition of PHA, the rate of blastformation in the former combination was 19.3% and 31.85% in the latter, showing a significant difference ($p=0.05$).

3. *Survival time of the homograft of the skin from F_1 mice to parent mice:*

In the transplantation of the skin graft from F_1 mice to the parent mice, the survival days proved to be 14+1.22 days in average, and in the F_1 to C3H mice transplantation it was 9.7 + 0.28 days in average, reveal-

ing a significant difference ($p=0.01$) between the two sets (Table 1).

Table 1. Percentage of Large and Intermediate Cells in Mixed Cultures of F_1 Cells and Parent Cells

Exp. Nos.	Strain Combination	Without PHA	With PHA
1	A + F_1	8.2 %	15.4 %
	C_3H + F_1	7.6 %	32.2 %
2	A + F_1	7.2 %	20.6 %
	C_3H + F_1	12.8 %	34.0 %
3	A + F_1	10.4 %	22.8 %
	C_3H + F_1	12.0 %	40.0 %
4	A + F_1	4.0 %	18.4 %
	C_3H + F_1	6.4 %	21.2 %
Average :	A + F_1	7.45 %	19.30 %
	C_3H + F_1	9.70 %	31.85 %
		p = 0.3	p = 0.05

SUMMARY AND DISCUSSION

It is said that 97 per cent of mouse lymphocytes are small lymphocytes (4) and hence those cells that show blastformation in tissue culture are thought to be small lymphocytes (5—11).

It is also considered that the greater is the genetic difference between the lymphocytes in mixed culture, the greater is the extent of blastformation. However, the lymphocytes irradiated with a large dose of Co^{60} and F_1 cells would theoretically not respond to the target cells, lymphocytes and their parent cells (12) On the other hand, the findings that the blastformation in the combination of irradiated A cells + normal C3H cells is greater than in the reverve combination of normal A cells + irradiated C3H cells, and that in F_1 cells + C3H cells is greater than in the combination of F_1 cells + A cells indicate that in either case, C3H cells with less histocompatibility antigens react more potently on $H\text{-}2^a$ antigens of A cells possessing more abundant H-2 antigenic components. Furthermore, since the *in vivo* result shows that the homograft of skin transplanted from F_1 to C3H mice is rejected earlier than that transplanted from F_1 to A mice and this phenomenon is in a reverse correlation with the rate of blastformation in the mixed culture of lymphocytes, it suggests that the *in vitro* mixed lymphocyte culture test can be used for the histocompatibility

testing. It follows then, if we select from various prospective donor lymphocytes and treat them by irradiation or by some other means whereby they are made unable to undergo blastformation and by selecting those with the least blastformation we can have a proper donor. Hence it is obvious that the skin graft from (A × C3H) F_1 donor is better accepted by A mice than by C3H mice.

Since non-H-2 antigens apart from the H-2 histocompatibility should differ between A mice and C3H mice, it seems rather unreasonable to limit the blastformation and the survival of homotransplantation of the skin only to the difference in the H-2 antigens. Therefore, it is necessary to study to what extent the blastformation differs owing to the difference in non-H-2 antigens.

There are reports dealing with the mutual relationship between the difference in mouse H-2 antigens and blastformation as by DUTTON and FESTENSTEIN, all these studies are done on the basis of the incorporation of labeled thymidine into the nucleus at the time of mitosis. DUTTON (13) in his mixed cultures of spleen cells from various mice of inbred strains conducted for 54 hours reported that quite variations were found in the incorporation of labeled thymidine. FESTENSTEIN (14) likewise conducted mixed cultures of spleen cells from various inbred mice for 5 days to determine blastformation of the culture cells on the basis of the extent of C^{14}-thymidine incorporation, but reported that there could be observed no significant correlation between the difference in H-2 antigens and the extent of response. However, none of them used PHA in their culture medium, hence the resultant blastformation was poor as to reflect sufficiently the differences in the H-2 antigens. It seems obvious that PHA enhances the specific reaction on the histocompatibility antigen of the cells.

CONCLUSION

In the mixed tissue culture of mouse lymphocytes with addition of PHA the rate of the appearance of large and intermediate cells increases markedly, but which side of the two cell groups have reacted stronger remains obscure. In order to solve this problem, mixed cultures were conducted in such a way that only one cell group of the two would react. Namely, one cell group was exposed to Co^{60}-irradiation (Table 2) prior to the culture and cultured with another viable cell group (F_1 test group, Table 2) to see the percentage of the appearance of large and intermediate cells. Simultaneously, the skin homograft from respective donor mouse

was transplanted to each other and the survival days of each skin graft were compared. As a result it has been shown that the percentage of blastformation and the survival time of the skin transplant in each group prove to be in an inverse relation.

The results of these mixed cultures indicate that the extent of blastformation reflects significantly the difference in H-2 histocompatibility antigens.

Table 2. Percentage of Large and Intermediate Cells in Mixed Cultures of Irradiated Cells and Untreated Allogeneic Cells

Exp. Nos.	Strain Combination	Without PHA	With PHA
1	[A] + C3H	7.0 %	26.2 %
	A + [C3H]	4.2 %	20.2 %
2	[A] + C3H	6.2 %	21.0 %
	A + [C3H]	8.0 %	15.4 %
3	[A] + C3H	8.2 %	20.4 %
	A + [C3H]	6.2 %	12.2 %
4	[A] + C3H	6.6 %	34.2 %
	A + [C3H]	3.0 %	24.6 %
Average :	[A] + C3H	7.00 %	27.95 %
	A + [C3H]	5.35 %	18.10 %
		p=0.2	p=0.05

[ ] : denotes irradiated cells.

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