Acta Medica Okayama

Volume 23, *Issue* 3 1969 *Article* 3

JUNE 1969

Identification of mouse H-2 antigens by mixed lymphocyte culture in the presence of PHA. 3. Blastformation of mouse lymphocytes according to the difference in non- H-2 antigens

Shoken Kaneda*

*Okayama University,

Identification of mouse H-2 antigens by mixed lymphocyte culture in the presence of PHA. 3. Blastformation of mouse lymphocytes according to the difference in non- H-2 antigens*

Shoken Kaneda

Abstract

It has been demonstrated that by the mixed cultures in the presence of PHA the combination of those cells whose H-2 antigens differ from each other shows a higher rate and more significant difference of blastformation than in the combination where non-H-2 antigens differ (Table 1). The blastformation observable in the combinations where non-H-2 histocompatibility antigens or sex.linked antigens are weaker, is not, so marked as the difference seen of the blastformation in the case with H-2 isoantigens. This in vitro lymphocyte stimulation test can be applied to the histocompatibility test in the combinations of strong H-2 antigens.

*PMID: 4242842 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **23**, 203—207 (1969)

IDENTIFICATION OF MOUSE H-2 ANTIGENS BY MIXED LYMPHOCYTE CULTURE IN THE PRESENCE OF PHA
III. BLASTFORMATION OF MOUSE LYMHOCYTES ACCORDING TO THE DIFFERENCE IN NON- H-2 ANTIGENS

Shoken KANEDA

Department of Surgery, Okayama University Medical School, Okayama, Japan (Director: Prof. S. Tanaka)

Received for publication, March 20, 1969

In the mixed cultures of mouse lymphocytes with different H-2 antigens conducted in such a way that only one cell group in the combination of the two is made to react, it has been shown that the blastformation of these small lymphocytes results from lymphocytes reacting more potently upon the other lymphocytes having H-2 antigen lacking inthemselves, and the lymphocyte stimulation test *in vitro* is an excellent one for the histocompatibility test in making H-2 antigens of the two cell groups match with each other in order to identify the cells with a stronger H-2 antigens. However, supposing if the blastformation should occur in the same degree as in the case against such antigens as non-H-2 histocompatibility antigen and sex-linked antigen which are the so-called weak histocompatibility antigens, the blastformation *in vitro* can no longer be suitable test of histocompatibility. In other words, such strains of lymphocytes that show strong blastformation can no longer be excluded as an unsuitable recipient.

By the mixed cultures of human peripheral blood lymphocytes of close blood relatives the blastformation is already shown to be weaker than in the mixed cultures of similar lymphocytes of non-related persons (1). While there are dissenting opinions (2, 3) and no concrete genetic basis for it has been established yet, such a phenomenon seems to reflect one of genetic factors. We performed repeated mixed cultures of the mouse lymphocytes with the so-called weak histocompatibility antigens, using the inbred mice whose gene is distinct but differing in their non-H-2 antigens or sex-linked antigen, though H-2 alleles are the same. As a result we found clearly that the extent of blastformation is less marked in the cases of the lymphocytes with weaker histocompatibility antigens. This report deals with our findings on this study.

MATERIALS AND METHODS

The methods and materials used are identical with those in the previous paper, and they are omitted here.

Lymphocytes: Axillary and cervical lymph nodes were obtained from A ($H\text{-}2^a$) mice, C3H, CBA ($H\text{-}2^k$) mice all being adult mice of both sexes, and these lymph nodes were minced, strained through the filter, and lymphocytes so prepared served as the materials.

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

Antibiotics used was penicillin (Takeda Pharmaceutical Co.).

Culture methods: Both in the single and mixed cultures 100×10^4 cells/ml were used in each culture. The culture was conducted by the stationary method at 37°C for 72 hours. Specimens were prepared at certain intervals, stained with Mays-Giemsa stain, and the lymphocytes were classified according to their size: large 110 $\mu^2 >$, intermediate 110 $\mu^2 > > 56\ \mu^2$, and small 56 μ^2,

RESULTS

In every case the culture was carried out in the presence of 1 % PHA (v/v). The rate of blastformation (the appearance of large and intermediate cells) is as shown in Table 1. It was found that the rate of blastformation was:

Percentage of large and intermediate cells in mixed and unmixed cultures

Strain combination	Experiment No.	Percentage with phytohemagglutinin-M					
		1	2	3	4	5	Average
A + C3H		27.4	40.2	38.4	34.8	32.4	34.8
CBA + C3H		17.2	30.6	22.2	19.6	20.2	22.0
A♂ + C3H♂		50.2	24.2	26.2	20.4	32.4	30.7
A♂ + C3H♀		26.6	30.0	21.0	34.2	28.3	28.0
C3H♂ + C3H♀		18.5	16.4	14.4	18.8	20.4	17.7
C3H♂		12.0	14.2	11.8	14.6	10.4	11.6
A♀ + C3H♂		39.4	32.0	29.4	37.6	33.3	34.3
A♀ + C3H♀		30.6	35.4	46.2	24.8	40.2	35.4
A♂ + A ♀		20.2	24.4	25.6	17.6	20.8	21.7
A♀		16.0	18.8	20.6	15.0	13.4	16.8

1) In single strain culture 14.2 % in average, 11.6 % in the case of C3H cells and 16.8 % with A cells. 2) Mixed culture in the combination of the same strain but different sex: average was 19.7 %; 3) Hetero-

genous strain in the combination of the same sex: aveage was 33 %. 4) Heterogenous strain in the combination of the different sex: average was 31.17 %.

In other words, the blastformation was 1)<2), showing it to be somewhat greater in the combination of the same strain of opposite sex. In comparing the results of 2), 3) and 4), the order of blastformation is 2)< 3), 4), indicating that it is higher in the heterogenous combination. There can be recognized no difference in the blastformation between heterogenous-same sex combination and heterogenous-opposite sex combination. Furthermore, in comparing the heterogenous mixed cultures themselves, the combination like (CBA+C3H) where the difference lies in something other than H-2 antigen, and in the combination of (A+C3H) where H-2 differs, the rate of blastformation is higher in the combination where H-2 antigens differ.

SUMMARY AND DISCUSSION

It has been demonstrated that blastformation is difficult to observe in the combination where both groups of the cells in the mixed culture are of the so-called weak histocompatibility antigens and also the extent of blastformation is slight in the case two groups have a marked difference in H-2 antigens such as A mouse lymphocytes with abundant H-2 isoantigens against C3H mouse lymphocytes with little H-2 isoantigens. These facts seem to show gene-dosage effect. It is already demonstrated that the skingraft transplanted between mice whose antigens differ at more than two weak H loci is rejected earlier than the skin transplanted between the mice whose antigens differ only at one H locus. Even in the cases of mice with H-2 isoantigens such a dosage effect (additive effect) is observable (6, 7), GRAFF *et al.* (5) performed skin graft transplantation experiments by using various oncogenic resistant lines of mice (males and females) as well as various combinations where antigens differed at 2, 3, 4, or weaker H loci using non-H-2 or sex-linked histocompatibility antigens, and also the combinations where antigens differed both at H-2 locus and non-H-2 locus, and observed the survival time of homo-skin grafts. They found that in the combination where H-2 locus was the same but differed at non-H-2 locus the weak H locus showed additive effect, and the greater the difference the greater was the transplantation immunity, and as such a difference grew more marked, the skingraft is rejected around 12 days, just as in the case of skingraft with antigens differing at H locus.

The potency of histocompatibility antigens depends upon the extent

of immune response induced by the antigen, and in the skin homografts between two strains whose antigens differ at H-2 locus the survival of the skin grafts is 8--10 days, but in the cases where antigens differ only at H-1 and H-3 (H-2 being the same), such skin graft survives several tens or even 100 days. KLEIN (8) has demonstrated that the majority of individual non-H-2 antigens act as a weak antigen as judged from the numbers of survival days of the skin homografts. It may be said that a strong histocompatiblity locus means a complex locus that controls many isoantigens, and the weak locus is the site where a small number of isoantigens are gathered.

It appears that for the induction of blastformation in lymphocytes there should be a difference in a considerable number of histocompatibility antigens as well as the difference in more than one H-2 isoantigens. As postulated by the so-called clonal theory of BURNET, the presence of lymphocytes which are destined to give rise to blastformation in response to antigens, brings about additive effect, and this phenomenon may be interpreted as the stronger the histocompatibility antigens of the lymphocytes the greater is the rate of blastformation, and conversely, the weaker is such histocompatibility antigens, the lesser the blastformation. The fact that, contrary to expectation, the combination of non-H-2 antigen yielded the strongest response in the mixed cultures of mouse lymphocytes as observed by FESTENSTEIN (9) using the C^{14}-thymidine-incorporation as the criterion, may somehow be explained by the gene-dosage effect.

In their mixed cultures of human lymphocytes in which there was a derangement of 4 antigens, 1, 2, 3, and 7 belonging to the major histocompatibility loci and those without such a derangement, IVANJI *et al.* (10) observed a higher rate of blastformation in those with the derangement but in those without such derangement, that is, the blastformation corresponding to those of strong antigens and the combination of different lymphocytes with weak antigens, the blastformation was less. Among numbers of histocompatbility testings the most feasible ones that do not sensitize the recipient nor give pain to either recipient or donor are two tests: namely, the mixed lymphocyte culture test and the serological test by the use of human antiserum. However, since the results by these two tests concert with one another (10, 11), the mixed lymphocyte culture test, provided that the major histocompatibility antigens as well as the weak histocompatibility antigens are contained numerously, would serve as the criterion for determining the blastformation, hence it would be sufficiently useful as än exclusion test of the donor.

CONCLUSION

It has been demonstrated that by the mixed cultures in the presence of PHA the combination of those cells whose H-2 antigens differ from each other shows a higher rate and more significant difference of blastformation than in the combination where non-H-2 antigens differ (Table 1).

The blastformation observable in the combinations where non-H-2 histocompatibility antigens or sex-linked antigens are weaker, is not so marked as the difference seen of the blastformation in the case with H-2 isoantigens.

This *in vitro* lymphocyte stimulation test can be applied to the histocompatibility test in the combinations of strong H-2 antigens.

REFERENCES

1. CHALMERS, D. G. *et al.*: Immunologically stimulated human peripheral blood lymphocytes *in vitro*. *Int. Arch. Allergy* **30**, 177, 1966
2. RUBIN, A. C. *et al.*: Histocompatibility and immunologic competence in renal homotransplantation. *Science* **143**, 815, 1964
3. ELVES, M. W. and ISRAELS, M. G. G.: Lymphocyte transformation in cultures of mixed leucocytes. A possile test of histocompatibility. *Lancet* **1**, 1184, 1965
4. MCKHANN, C. F.: Additive effect of multiple weak incompatibilities in transplantation immunity. *Nature* **201**, 937, 1964
5. GRAFF, R. J., SILVERS, W. K., BILLINGHAM, A. E., HILDEMANN, W. H. and SNELL, G. O.: The effect of histocompatibility antigens. *Transplantation* **4**, 605, 1966
6. KOKUMAI, Y.: Humoral and cellular reactions against the transplanted cells. *Acta Medicinae Okayama* **20**, 235, 1966
7. HILDEMANN, W. H. and MEDAWAR, P. B.: Relationship between the transplantation immunity and formation of humoral isoantibodies in mice. *Immunology* **2**, 44, 1959
8. KLEIN, J.: Strength of Histpcompatibility Genes in Mice in Histocompatibility Testing. (CURTORI, E. S., MATTIUZ, P. L. and TOSI, P. M,) Munksgard, Copenhagen pp 21—29
9. FERSENSTEIN, H.: Antigenic strength investigated by mixed culture of allogenic mouse spleen cells. *Ann. N. Y. Acad. Sci.* **129**, 567, 1966
10. IVANYI, D., RYCHLIKOVA, M., SASPOTES, M., IVANYI, P., DAUSSET, J.: Leukocyte antigens and the mixed lymphocyte culture reaction. *Vox. Sanguinis* **12**, 186, 1967
11. BACH, F H. and AMOS, D. B.: Hu-1: Major histocompatibility locus in man. *Science* **156**, 1506, 1967