Plaque-Forming-Cell Response to H-2 Antigens of Mice[1] (35235)

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In order to study immune responses against surface antigens of nucleated cells, we have recently developed a procedure for detection of plaque-forming cells (PFC) in which xenogeneic as well as allogeneic thymus cells were employed as immunizing and target antigens (1, 2). The previous studies with mice stimulated by allogeneic thymus cells revealed that in the majority of strain combinations, PFC produced antibodies to θ antigens of murine thymocytes. Our attempts to demonstrate PFC producing antibodies to H-2 antigens failed even when spleen cells which are rich in these antigens were employed for immunization and as a target. The present report describes experiments in which murine lymphoblasts were successfully employed as target cells for detection of PFC producing antibodies to H-2 antigens.

Materials and Methods. Murine lymphoblasts. The lymphoblast cell line, L5178Y, was kindly supplied by Dr. L. A. Manson of the Wistar Institute, Philadelphia. The cell culture was established and cloned by Fischer (3) from a lymphocytic leukemia developed by a DBA/2 mouse treated with methylcholanthrene (L. A. Manson, personal communication). Manson *et al.* (4) have shown that L5178Y cells still contain H-2 antigens of the DBA/2 strain. The cells were maintained *in vivo* as an ascites tumor by the successive weekly transfers (0.1 ml of ascitic fluid, approximately 10^7 cells) in DBA/2J mice. To prepare target cells, the ascites cells were harvested 6–7 days after transplantation of the tumor. The cells were centrifuged at 1000 rpm for 10 min in a refrigerated centrifuge, resuspended in 4 vol of cold medium 199 and counted in a hemocytometer. Plaque assays with immune spleen cells were carried out as described previously (1, 2). Briefly, to each tube with 0.3 ml of 0.7% Bacto agar (Difco Laboratories, Detroit, Michigan) dissolved in medium 199 and containing 0.5 mg of DEAE-dextran/ml was added 0.05 ml of a suspension containing 1×10^7 L5178Y cells and 0.05 ml of a suspension containing a suitable number (not exceeding 4×10^6) of immune spleen cells. The mixture was immediately spread on a microscopic slide coated previously with 0.1% agar. The slides with their surfaces covered with the medium were incubated for 1 hr at 37° in a CO_2 incubator. The medium was replaced with rabbit complement diluted 1:10 and the incubation was continued for 1 hr. Thereafter the slides were rinsed in saline and dried by a fan at room temperature. Dried preparations were fixed in 95% ethanol for 30 min and rinsed in tap water to remove salts. The slides were then examined for plaques against strong light using a magnifying lens. Triplicate slides were used for an assay with each spleen.

Results. Mice of five inbred strains with different H-2 alleles were injected intravenously, each with 4×10^7 spleen cells of female DBA/2 mice. Six days after the stimulation, immune spleen cells of the host animals were assayed for PFC using L5178Y lymphoblasts as target cells (Table I). Host mice of all the strains under investigation developed PFC except for one strain, BALB/c which shares the H-2^d allele with the donor strain, DBA/2. The strains of mice which demonstrated PFC in this experiment showed PFC response of a similar mag-

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TABLE I. Appearance of PFC in Mice of Various Strains After Stimulation with Spleen Cells of DBA/2 Strain (H-2^d).[a]

Strain	H-2	No. of animals	No. of PFC/spleen	
			Arithmetic mean	Range
A	*a*	7	802	200–2170
C57BL/6	*b*	8	6850	1060–17600
BALB/c	*d*	3	<20	<20
C3H	*k*	8	1160	610–1980
Inbred Swiss-Webster	?	9	9420	3800–13700

[a] Host animals were given an intravenous injection of 4×10^7 DBA/2 spleen cells. The assay with L5178Y target cells was performed 6 days after the stimulation.

nitude when they were stimulated with BALB/c spleen cells. As expected, DBA/2 mice stimulated with BALB/c spleen cells failed to develop PFC. As shown in Fig. 1, PFC appeared in the spleen of C57BL/6 mice 2 days after the intravenous injection of 4×10^7 DBA/2 spleen cells. The maximum PFC numbers of 10,030 per spleen were observed on day 6. Virtually no PFC were detected when spleen cells of normal C57BL/6 mice were examined in a similar way against the same target cells.

PFC could also be detected in the spleen of C3H mice stimulated by full-thickness skin grafts of DBA/2 origin, indicating that skin contains antigens involved in the PFC assay. The peak number of 2660 PFC/spleen was observed on day 12 although the numbers varied widely among individual hosts. Interestingly, in all tests performed between 2 and 14 days after transplantation, the numbers of PFC per 10^7 nucleated cells were considerably lower in regional lymph nodes than in the spleen.

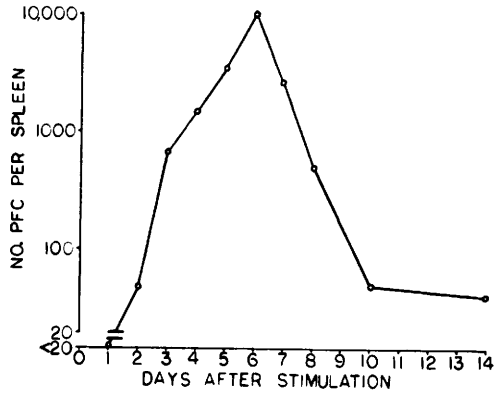

FIG. 1. C57BL/6 mice were stimulated with an intravenous injection of 4×10^7 DBA/2 spleen cells. PFC assays of host spleen cells were done using L5178Y cells as target. Each point on the curve represents arithmetic mean value obtained in 4 to 10 animals.

In subsequent experiments, congenic strains of mice were employed to study further whether the observed PFC response was directed against antigens controlled by H-2 locus. As shown in Table II, the PFC response was also noted in those strain combinations in which host and donor differed only at H-2 locus.

Discussion. The foregoing data demonstrated that PFC which produced antibodies against H-2 antigens were detected by means of the described procedure. L5178Y ascites tumor used as a source of target cells consists of homogeneous population of cells which can readily be dispersed in agar in the form of single cells. These features are probably important for the successful demonstration of the PFC. As mentioned previously L5178Y retained the H-2 antigens of the DBA/2 strain.

The experiments on congenic strains shown in Table II (groups 1, 2, and 3) indicated that a majority of PFC, if not all, arose in response to H-2^d antigens. Stimulation of host animals with H-2^a antigens also resulted in the PFC response (group 5) and the numbers of PFC were almost as high as in the stimulation with H-2^d. These results are consistent with the fact that H-2^a has all of the H-2^d specificities except for "31" (5). H-2^a mice were also used as host animals (group 6 and 7). As could have been expect-

TABLE II. Appearance of PFC in Host–Donor Combinations Employing Congenic Strains of Mice.[a]

Group	Host	Donor	H-2		Difference in non-H-2	No. of animals	No. of PFC/spleen	
			Host	Donor			Arithmetic mean	Range
1	C57BL/10	DBA/2	*b*	*d*	+	4	1240	370–2400
2		B10.D2	*b*	*d*	—	9	1260	400–2800
3	B10.D2	DBA/2	*d*	*d*	+	9	36	20–300
4		C57BL/10	*d*	*b*	—	9	<20	<20
5	C57BL/10	B10.A	*b*	*a*	—	6	750	110–3010
6	B10.A	C57BL/10	*a*	*b*	—	8	<20	<20
7		B10.D2	*a*	*d*	—	4	313	27–800

[a] Host animals were given an intravenous injection of 4×10^7 donor spleen cells. The assay with L5178Y target cells was performed 7 days after the stimulation.

ed, no PFC response was observed after stimulation with H-2^b cells and rather weak response was elicited with H-2^d cells. The PFC found in the latter strain combination might have been producing antibodies of the "31" specificity. Demonstration of PFC in host animals stimulated with allogeneic skin graft provided additional evidence that histocompatibility antigens were involved in the observed PFC response.

The use of lymphoblast cell line as a source of target cells in plaque assay offers a way of studying immune responses against histocompatibility antigens at cellular level. Although this system does not detect cells which may be immunologically active but do not release antibodies, it does provide a quantitative measure of immune responses by enumerating individual antibody forming cells. Furthermore, the system should facilitate studies of the relationship of two cell populations in terms of their origin and their immunological function in transplantation immunity.

Summary. Lymphoblast cell line L5178Y of DBA/2 mouse origin was used as a source of target cells for a cytolytic plaque assay. Plaque-forming cells were demonstrated in spleens of mice injected with allogeneic cells or grafted with allogeneic skin. From the results obtained in various inbred strains, it could be concluded that the major immune response measured in this study was directed against H-2^d antigens.

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