Studies of Formation of Transplantation Antibodies in Mouse Parabiosis[1] (35830)

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In a few reports dealing with serological aspects of parabiosis, formation of humoral antibodies to the partner's antigens has been shown (1–3). Moreover, evidence for transfer of such antibodies from one partner to another has been presented (2). The role played by these antibodies in pathogenesis of parabiotic intoxication is not fully understood (4, 5). Having in our hands a method which permits enumeration of cells producing antibodies directed against surface antigens of nucleated cells (6–8), we considered it worthwhile to study kinetics of emergence of cells forming antibodies to the partner's histocompatibility antigens in the course of parabiosis. The present report describes preliminary experiments along this line.

Materials and Methods. Inbred mice of strains C3H/HeJ, AKR/J, DBA/2J, C57BL/6J, C57BL/10J were purchased from Jackson Laboratories, Bar Harbor, Maine. They will be referred to henceforth as C3H, AKR, D2, B6, and B10, respectively. Inbred Swiss albino mice RR and F_1 hybrids have been raised in animal facilities of this University.

Coelomic parabiosis was performed between mice 8–10 weeks old. In most, but not all, experiments both partners were of the same sex. The number of cells producing antibodies against transplantation antigens in the spleens of parabionts was determined using previously described methods (6–8). The principle of the method is that antibody producing cells incorporated into the agar along with appropriate target cells cause a localized, complement dependent lysis of target cells. The cytolysis can be seen in form of plaques and therefore antibody producing cells are referred to as plaque-forming cells (PFC).

In the first experiment in which thymocytes were used as a target, PFC producing antibodies against thymic θ antigens were detected (7). Mice carrying θ-C3H antigen, C3H or RR were parabiozed with AKR mice carrying θ-AKR antigen. C3H were also joined with F_1 hybrids carrying both antigens. These hybrids were obtained by mating of AKR mice with mice carrying θ-C3H antigen: C3H, B6, or RR. Spleen cells of homozygous partners were assayed on thymocytes of partner strain as well as on thymocytes of the animal's own strain. Spleens of the F_1 partners were assayed only on thymus cells carrying θ-AKR antigen.

In the second experiment parabiosis was performed between C3H and either D2 or $(D2 \times C3H)F_1$ or $(D2 \times B10)F_1$. In this case the response of C3H mice against $H2^d$ antigens was studied using, as a target, murine lymphoblast cell line L5178Y which shares $H2^d$ antigens with D2 mice (9, 10). The same target cells were used to investigate spleens of D2 and F1 mice for PFC involved in production of antibodies against the animal's own antigens ($H2^d$). Unfortunately, suitable target cells carrying $H2^k$ antigens were unavailable, thus PFC producing antibodies against H2 antigens of C3H partner could not be studied.

In an additional experiment, AKR and D2 mice have been parabiozed and spleen cells of AKR partner were assayed on C3H thymocytes to detect the response against θ-C3H antigen and on lymphoblast cell line to detect response against $H2^d$ antigens, both carried by D2 partner.

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Each experimental group consisted of 4–8 pairs and each spleen was assayed in triplicate.

Cytolytic antibodies in sera of experimental animals were detected by means of the "spot test" described in a previous communication (7). Briefly, droplets containing approximately 20 μl of serum at twofold serial dilutions were placed on the agar layer containing appropriate target cells. Thymus cells were employed to detect antibodies to θ antigens. Antibodies to $H2^d$ antigens were detected on L5178Y lymphoblasts. The antibody titer was expressed as the reciprocal of the highest dilution of serum that elicited distinct lysis of target cells after addition of complement.

As a source of complement for both plaque assays and spot tests fresh or preserved (—20°) normal rabbit serum, diluted 1:10 was used.

For control experiments C3H mice were immunized by intravenous injection of 5×10^7 thymus cells or spleen cells. The spleens of immunized animals were assayed for presence of PFC by similar procedures as spleens of parabionts.

Results. Parabiosis of C3H with AKR mice resulted in appearance of PFC producing antibodies against the θ antigen of the partner. Figure 1 shows the number of PFC in spleens of C3H and AKR mice tested against the partner's thymocytes at various times during parabiosis. As shown, C3H mice produced a peak number of 10^4 PFC/spleen while AKR mice had only 2.6×10^2 PFC/spleen. Similar experiments were performed with another θ-C3H strain, RR mice. In this parabiosis, RR partners produced 0.8×10^4 PFC/spleen while AKR partners produced only 0.8×10^2 PFC/spleen.

In spleens of C3H mice parabiozed with F_1 hybrids carrying one θ-AKR allele, the number of PFC was 2.5–70 times lower than the number of PFC in spleens of C3H parabiozed with AKR mice (Table I). Similar differences were found among numbers of PFC in C3H mice which were immunized iv with AKR or F_1 thymus cells.

In testing spleen cells of C3H and RR parabionts on C3H thymus cells and spleen

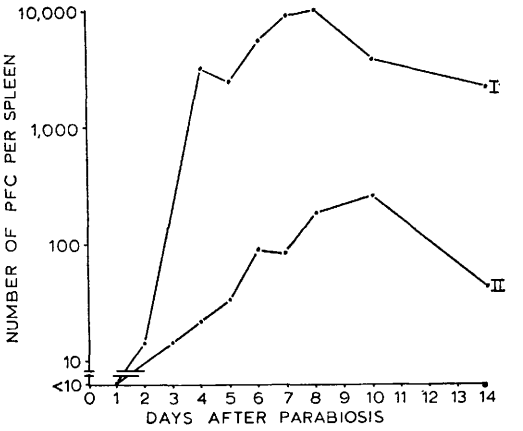

FIG. 1. Number of PFC in spleens of C3H and AKR mice at different times during coelomic parabiosis between these mice: (I) PFC in C3H spleens revealed on AKR thymus cells; (II) PFC in AKR spleens revealed on C3H thymus cells. Each point represents arithmetic mean value from 5–6 animals.

cells of AKR and F_1 parabionts on AKR thymus cells, in some individuals very few PFC could be found; the numbers never exceeded 30 PFC/spleen.

Figure 2 shows the numbers of PFC producing antibodies against $H2^d$ antigens, which were counted in spleens of C3H mice parabiozed with D2 mice. As shown, 5 days after parabiosis a number of 5×10^3

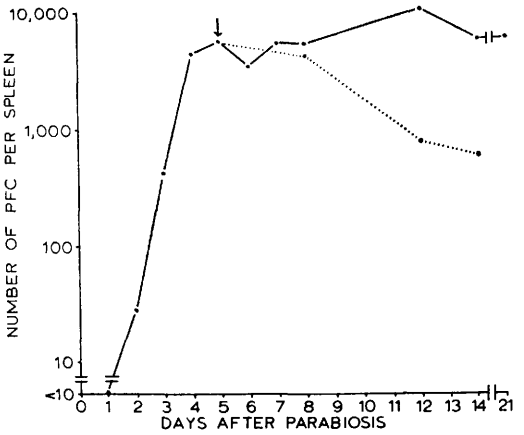

FIG. 2. Number of PFC in spleens of C3H mice at different times during coelomic parabiosis with D2 mice. Target cells, L5178Y lymphoblast cell line. (—) PFC in spleens of C3H mice while in parabiosis; (. . .) PFC in spleens of C3H mice separated from D2 partners on day 5 (arrow). Each point represents arithmetic mean value from 5–6 animals.

TABLE I. Number of PFC in Spleens of C3H[a] Mice Parabioted with Partners Homozygous or Heterozygous in Respect of θ-AKR or $H2^d$ Antigens.

Partner with which C3H mice were parabioted	Alleles		No. of PFC/spleen of C3H mice	
	H2	θ	Mean	Range
			C3H spleen assayed on AKR thymocytes on day 7 of parabiosis	
AKR	k/k	AKR/AKR	8762	3850–19400
(AKR × C3H) F_1	k/k	AKR/C3H	123	33–390
(AKR × B6) F_1	k/b	AKR/C3H	3368	850–6400
(AKR × RR) F_1	k/?	AKR/C3H	514	48–1810
			C3H spleen assayed on murine lymphoblasts L5178Y on day 5 of paraboisis	
D2	d/d	C3H/C3H	6537	2300–9800
(D2 × C3H) F_1	d/k	C3H/C3H	12312	4250–24700
(D2 × B10) F_1	d/b	C3H/C3H	12937	8700–17100

[a] H2 alleles: k/k; θ alleles: C3H/C3H.

PFC/spleen was found and, with little variations, this number persisted until 3 weeks of parabiosis. When mice were separated surgically on day 5 the number of PFC in their spleens decreased rather quickly. Interestingly enough, parabiosis of C3H mice with F_1 hybrids heterozygous at H2 locus and carrying $H2^d$ allele resulted in higher numbers of anti-$H2^d$ PFC than seen during parabiosis with homozygous animals (Table I). Assaying of spleens of D2 mice and F_1 parabionts on target cells carrying $H2^d$ antigens revealed only very few PFC (less than 30/spleen) in some individuals. As stated previously, because of unavailability of proper target cells PFC responses against $H2^k$ antigens of C3H mice could not be studied.

In C3H mice immunized by a single injection of 4×10^7 spleen cells, peak numbers of 10^3 PFC/spleen could be scored, a number about five times smaller than the one observed in parabiosis.

In spleens of AKR mice parabiozed with D2 mice carrying θ-C3H and $H2^d$ antigens PFC producing antibodies to both antigens could be scored. Such spleens contained 7×10^2 anti-θ-C3H PFC and 1.6×10^3 anti-$H2^d$ PFC.

Figure 3 presents the results of titration of sera of parabiozed animals for cytolytic antibodies. In sera of C3H mice parabiozed with AKR, antibodies lysing AKR thymocytes were detectable on day 2 and reached the highest titer of 2^{10} on day 10. Beginning from day 8 of parabiosis, sera of these animals contained also antibodies lytic for C3H thymocytes which reached peak titer of 2^4 on day 10. In sera of AKR mice, antibodies lytic for C3H thymocytes appeared on day 7 of parabiosis and reached peak titer of 2^5 on day 10. Interestingly enough, antibodies to AKR thymus cells appeared in sera of AKR

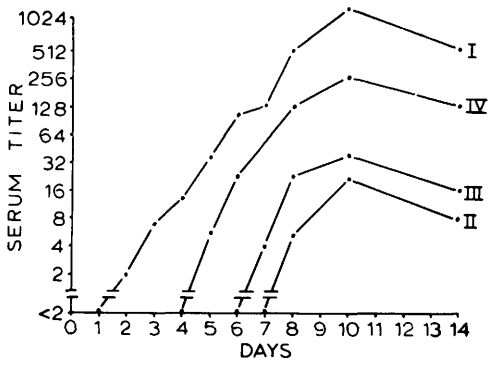

FIG. 3. Titer of cytolytic antibodies in sera of C3H and AKR mice at different times during coelomic parabiosis between these mice. Antibodies in C3H sera detected on AKR (I); and C3H (II) thymus cells. Antibodies in AKR sera detected on C3H (III); and AKR (IV) thymus cells. Each point represents geometric mean value from titers of 2–3 samples of serum pooled from 2–3 animals.

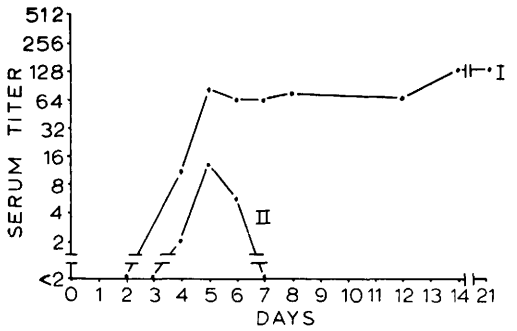

FIG. 4. Titer of cytolytic antibodies in sera of C3H (I); and D2 (II) mice at different times during parabiosis between these mice. Target cells, L5178Y lymphoblast cell line. Each point represents geometric mean value from titers of 2–3 samples of pooled sera from 2–3 animals.

mice already on day 5 and reached a titer as high as 2^8 on day 10.

Figure 4 shows the results of experiments in which sera of C3H and D2 parabionts were tested for the presence of antibodies against $H2^d$ antigens. As shown, sera of C3H partners contained anti-$H2^d$ antibodies which could be demonstrated on day 4 and reached a titer of 2^6 on day 5; this titer persisted with little variation to the end of observation, day 21. Sera of D2 partners contained anti-H-2^d antibodies only between day 4 and 6 of parabiosis; maximum titer was 2^4.

Discussion. Reciprocal antibody formation was found when adult mice were connected by coelomic parabiosis. Depending on strain combination, response against thymic θ antigens, H2 histocompatibility antigens, or both, could be detected.

Certain differences could be noted when PFC responses resulting from parabiosis were compared with responses elicited by a single iv immunization with thymus cells (7). In parabiosis duration of anti-θ response persisted longer than response elicited by iv immunization. While C3H mice responded with similar numbers of PFC after parabiosis and iv immunization, AKR mice revealed evidently lower response in parabiosis. One can suppose that this resulted from the action of antibodies to θ-AKR which were produced by the C3H partner and which crossed into the AKR partner. These antibodies might have suppressed immune responses of the AKR mouse by acting on thymus-derived lymphocytes, an effect similar to the one exerted by an antilymphocyte serum. Analogous suppression of immune response did not seem to occur in the C3H partner. This may be explained by the fact that the normal ability of AKR mice to produce PFC against θ-AKR antigen is approximately 10 times lower than the ability of C3H mice to produce PFC against θ-AKR antigen (7).

Parabiosis of C3H with D2 mice resulted in significantly higher PFC response of C3H partners to $H2^d$ antigens than single iv immunization. The number of PFC in spleens of C3H parabionts connected with D2 mice, after reaching the peak on day 5, stayed on this level until day 21 of parabiosis. The continuous stimulation seemed to be responsible for this since separation of animals lead to a quick decline of the response.

Partners heterozygous in respect to θ antigen evoked significantly lower number of PFC in C3H parabionts than did the homozygous partners. This could be logically explained by the dose effect of the antigen. Interestingly, partners heterozygous at H2 locus induced in C3H mice higher response to $H2^d$ than homozygous partners. We cannot offer any satisfactory explanation for these findings. It may be stated, however, that in experiments which have not been described in results, we found that in other strain combinations differing in H2 allele the homozygous partner elicited also weaker PFC response than the heterozygous partner.

In parabiosis between θ-different as well as between H2-different partners, in some animals very few PFC producing antibodies against animal's own antigens could be found. It is reasonable to assume that such PFC developed in the lymphatic tissue of the partner and their presence indicated some exchange of PFC between parabiozed animals. Actually, one would expect a substantial degree of cell transfer only from the parental partner to the F_1 partner. It was shown (3, 11) that there is very small if any transfer of lymphoid cells from F_1 to parental partner or between allogeneic homozygous partners.

The PFC response was accompanied by presence of humoral antibodies directed against antigens of the partner. The titer of these antibodies paralleled the number of PFC found in spleens of the animals. The results obtained showed that these antibodies were exchanged between partners which is evident from the fact that there was correlation between titers of a given antibody found in sera of both partners (Fig. 3). Since only negligible number of PFC was exchanged between parabionts it is reasonable to exclude the transferred cells as a source of antibodies directed against the host's antigen.

Finally one wonders what is the role of antibodies to histocompatibility antigens in pathogenesis of parabiotic intoxication (3–5, 12–14). To explain the mechanism of parabiotic intoxication at least two hypotheses were proposed but neither one was well supported experimentally. Some investigators (4, 12–15) believe that anemia of one partner is responsible for its death which is followed by the death of the other partner unless it is separated soon enough. Other authors (16–18) assume that cell-mediated graft-versus-host reaction is the major cause of pathological symptoms. The present studies showed clearly production of cytolytic antibodies which were transferred from one partner to another. On the other hand, only negligible transfer of antibody producing cells could be detected. The significance of such cell transfer cannot be judged from the present findings but it seems that such transfer occurred only incidentally since the number of transferred PFC did not follow the kinetics of PFC in spleens of animal from which they originated. It is reasonable to assume that such a transfer does not reflect the degree of immune attack of one partner against the other. Obviously, the present studies neither exclude nor support the existence of cellular chimerism in parabiosis (11, 13, 19, 20).

Summary. Coelomic parabiosis between mice differing in θ and/or H2 antigens was produced. Spleens of parabionts contained plaque-forming cells involved in the production of antibodies to the partner's antigens. No significant exchange of PFC between partners was noted. Sera of parabionts contained lytic antibodies to the partner's antigens as well as the animal's own antigens. Data supporting the mutual exchange of antibodies were presented. Evidence was obtained that anti-θ antibodies produced in one partner suppress immune response of the other partner whose circulation they entered.

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