

IMMUNOLOGICAL TOLERANCE

DISSOCIATION BETWEEN IN VIVO AND IN VITRO REACTIVITY IN PARABIOSED MICE*

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The mixed lymphocyte culture (MLC) reaction has been used as a means of assessing the state of tolerance between allogeneic cells within chimeric individuals (1-5). As such it has proven to be a valuable in vitro correlate of in vivo immune reactivity, but its exact relation to other types of immune reactivity, such as cell-mediated cytotoxicity, is not fully understood.

In this paper we report the use of the MLC reaction to assess an intriguing but long neglected observation on tolerance induction in *H-2* incompatible adult mice by parabiosis. In 1959 Rubin observed that the parabiosis of DBA/2-C3H/HeJ F₁ hybrids with DBA/2 parental mice led to an unexpectedly high long-term survival rate of the pairs (29/71), compared to other strongly histoincompatible strain combinations (6). He also pointed out that the DBA/2 member of the pair became tolerant to C3H skin grafts, although the grafts were chronically rejected 3-4 mo after the termination of parabiosis. We have confirmed his observations, and in addition have made the surprising discovery that both partners of the long-term DBA/2:DBA/2-C3H F₁ parabiotic union are fully reactive against C3H antigens in MLC.

Materials and Methods

Parabiosis.—The strains of mice used were DBA/2J, C3H/HeJ, and F₁ hybrids between the two strains, obtained from the Jackson Laboratories, Bar Harbor, Me. Seven 10-wk old DBA/2J female mice were joined in parabiosis to seven DBA/2-C3H F₁ hybrid mice of the same age and sex. The method used was identical to that described by Bunster and Meyer (7), except that wound clips were used instead of sutures for closing the skin. The parabiotic pairs were maintained on tetracycline (0.02% Terramycin, Pfizer, Chas., & Co., Inc., New York.) for 2 wk postoperatively, to prevent infection. One pair died 35 days postoperatively and another pair succumbed while being skin-grafted, at 10 wk after union. The rest survived indefinitely.

Skin Grafting.—The method of skin grafting used in this study was that of Bailey and Usama (8), which involves the grafting of tail skin. Skin grafts were placed on the DBA parabiotic partners 10 wk after parabiosis.

Mixed Lymphocyte Cultures.—MLC's were performed on the parabiosed mice approximately 3 mo after surgery. The technique for performing MLC's was essentially that of Adler, et al. (9) as modified by Phillips, et al. (10). Harvesting was performed after 3 days in culture. Tritiated thymidine incorporation is expressed in this paper as an isotope incorpora-

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tion index, defined as

$$\frac{\text{cpm in mixed cultures}}{\text{cpm in both types of control cultures}}$$

where the value for each culture is the average of four replicate determinations.

RESULTS

Five pairs of age-matched DBA and DBA-C3H F_1 mice were parabiosed at 10 wk of age and the DBA partners were tail-grafted with C3H/HeJ, DBA/2, and C57Bl/10SnJ skin grafts at 20 wk. The partners were separated, and splenectomised at periods ranging from 14 to 19 wk from the start of parabiosis, and their splenic reactivity assessed in MLC.

Appearance.—In accordance with Rubin's observations the parabionts did not succumb to runtting disease, but appeared to be quite healthy. No overt signs of skin rejection were visible although, curiously, all the F_1 's developed a dark pigmented hood in the dorsal cranial fur which was not observable in unparabiosed mice of similar age. There was no obvious disparity between the parental and F_1 body weight.

Tail Grafts.—C3H and DBA grafts were retained on the DBA parabionts in every case, although the animals rejected the C57Bl/10 grafts in normal time. The parental strain grafts were still present 22 wk after separation although the C3H skin grafts had begun to show a lack of pigmentation characteristic of rejection. DBA controls rejected C3H skin grafts in normal time.

Spleen Size.—Gross changes in spleen size usually accompany parabiosis intoxication between histoincompatible strains. Splenomegaly followed by involution are the usual concomitants of chronic graft vs. host reactivity (11). The spleen weights of the DBA's (141 ± 18 mg) and F_1 's (121 ± 41 mg) were moderately affected by parabiosis, but did not show gross divergence from the norm in our colony (110 ± 35 mg). The DBA spleens of each pair were slightly larger than the F_1 's with one exception in which both spleens showed enlargement, a possible consequence of infection.

MLC Reactivity.—Fig. 1 presents the data obtained by reacting the spleens of both partners of the five parabiotic pairs with various control animals. In Fig. 1 *A* the reaction of the DBA parental strain animal used in parabiosis (marked with an asterisk) is compared to that of a normal unparabiosed DBA animal. The pattern of reactivity of the parabiosed DBA is indistinguishable from that of a normal DBA in that the parabiosed DBA does not react with a normal DBA, it reacts slightly with a normal F_1 , it reacts well with a normal C3H and also with a normal C57Bl/10. In each case the reactivity is indistinguishable from that of a normal DBA. This is somewhat surprising in view of the fact that the parabiosed DBA has been joined to an F_1 animal for a long time and is also bearing a C3H skin graft. Fig. 1 *B* displays the reactivity of the parabiosed F_1 (marked with an asterisk) compared to that of a normal

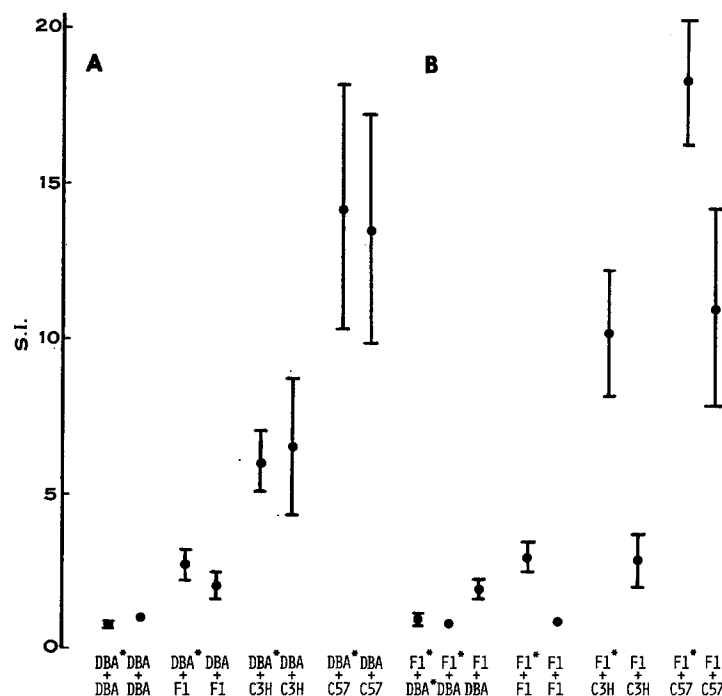


FIG. 1. MLC reactivities of DBA and DBA/C3H F_1 hybrid parabionts in two-way reactions with normal parental F_1 and 3rd party cells. The combinations are displayed on the abscissa. Parabiosed animals are indicated by an asterisk. The ordinate represents the stimulation index (S.I.) (see Materials and Methods). Each point represents the mean stimulation index (and the standard error) of five separate animals. (A) Comparison between DBA parabionts and normal DBA's. (B) Comparison between F_1 parabionts and normal F_1 's.

F_1 . The parabiosed F_1 does not behave like a normal F_1 , but as if it were a normal DBA. It does not react at all to a normal DBA animal, (nor to its own parabiont partner), it reacts to a normal F_1 , and it displays higher reactivity with C3H than does a normal F_1 . These results are best interpreted by assuming that the F_1 hybrid immune system has been taken over by cells derived from the DBA parent, although it is clear from what was said above that these cells are causing no obvious harm to the intact animal.

DISCUSSION

In the experiments reported here the MLC reactivities of the parabiosed F_1 hybrids are entirely in accordance with the concept that the F_1 spleen has been colonized by DBA cells. The reactivity of the parabiosed F_1 spleen cells to normal F_1 spleen cells, and the enhanced response with C3H spleen cells suggest that the F_1 spleen acts as a trap for circulating DBA lymphoid cells with anti-C3H specificity. The absence of chronic involution of the F_1

spleen indicates that cellular immunity is not expressed *in vivo*. Since DBA cells appear to colonize the F_1 , it is likely that the F_1 lymphocytes have invaded the DBA to some extent, as well. This would be compatible with the notion that chimerism is necessary to maintain tolerance. The waning of tolerance to the C3H skin graft following several months of separation most likely reflects the gradual elimination of F_1 cells from the DBA, although this has not been tested for here.

These experiments lead to the surprising conclusion that full MLC reactivity can exist in completely healthy (and hence tolerant) parabiosed mice differing at the major histocompatibility (*H-2*) complex. This indicates that the recognition phase of the allogeneic response can be clearly dissociated from the effector phase by the tolerogenic mechanism, with the strain combination employed here. The means by which this occurs is unknown, but may bear a relation to other experiments dissociating the two types of reactivity. Bach and his colleagues, for example, have physically separated the cells reactive in MLC from those capable of killer cell reactivity (12). Eijsvoogel et al. have described studies in which certain pairs of siblings who are HLA compatible by serological criteria give a positive MLC, but the cells from these cultures develop no killer cell activity *in vitro* (13). These two sets of experiments suggest that MLC reactivity and effector cell reactivity may be separable both at the cellular and at the genetic level. The experimental model described here may be useful in furthering such analysis because the tolerance mechanism active during parabiosis apparently distinguishes between the two types of reactivity, inactivating only effector cell activity. Whether this is accomplished by blocking the development of killer cells from those reacting in MLC remains to be seen. Experiments to examine this possibility are in progress.

The results also force the conclusion that tolerance should be defined operationally rather than mechanistically as has been common practice in the past. The parabiosed animals are tolerant by every conceivable criterion *in vivo* but still show full MLC reactivity *in vitro*. Concomitant cellular immunity and acceptance of grafted tissue is not unusual in kidney transplant situations where the kidney can apparently be protected by alloantibody (14). Also, Wilson et al. have demonstrated some MLC reactivity in a proportion of rats made tolerant to skin grafts by neonatal injection of allogeneic cells (15). Boyse and Old have shown, on the other hand, that X-irradiated adult mice can be made tolerant to transplantation antigens borne on chimeric lymphoid cells, but still reject skin syngeneic to the chimeric cell population (16). Also, lymphoid chimeras arising through placental anastomosis between dizygotic twins are frequently intolerant of skin grafts (17). These experiments as well as those described here, emphasize that transplantation tolerance is not an all or none phenomenon, and that the term tolerance should only be used in an operational manner to describe a specific and clearly circumscribed state of unresponsiveness rather than as a blanket term.

SUMMARY

Although most mouse strain combinations succumb to a lethal wasting disease when parabiosed across a strong histocompatibility (*H-2*) barrier, a high proportion of DBA mice parabiosed to DBA/C3H F₁ hybrids survive and appear healthy. DBA mice accept C3H skin grafts following parabiosis, and may therefore be considered operationally tolerant of C3H antigens. Nonetheless, spleen cells from long-term DBA and F₁ parabionts give normal and enhanced responses, respectively, to C3H antigens in mixed lymphocyte culture (MLC). This indicates that the tolerance mechanism can distinguish between MLC recognition reactivity and in vivo effector reactivity, and that the former can therefore exist in the absence of the latter.

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