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IN VIVO LYMPHOCYTE TRANSFORMATION INDUCED BY H-2D AND H-2K COMPONENTS OF THE H-2 LOCUS

By Diana M. Popp

Reports from several laboratories have shown that differential immune reactivity can be induced by different portions of the H-2 locus.¹⁻³ By studying in vivo lymphocyte transformation induced by host antigens it has been possible to show that the cellular response to a non-H-2 antigen is delayed and qualitatively different from the response to H-2 antigens.^{4,5} In the present study, in vivo lymphocyte transformation was induced by incompatibilities at different portions of the H-2 locus in an attempt to learn more about the cellular basis of differential reactivity induced by different H-2 determinants. The immunogenetic effect of these incompatibilities was assessed by following the antigen-dependent proliferation of donor cells in the spleen.

MATERIALS AND METHODS

Mouse strains used in these experiments were the congenic resistant pair C57BL/10Sn(B10) and C57BL/10.A(B10.A) and the H-2 recombinant lines

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C57BL/10.A(2R) and C57BL/10.A(5R). The linear order of the H-2 specificities that distinguish these strains⁶ is presented in simplified form in Fig. 1. The 2R and 5R lines resulted from crossovers between H-2^a and H-2^b at the positions indicated by arrows;⁶ 2R received the portion of the genome to the left of the arrow (H-2D) from H-2^b of B10 and the portion to the right of the arrow (H-2K) from H-2^a of B10.A; 5R received H-2D genetic information from H-2^a and the H-2K segment from H-2^b. Therefore, when 2R or 5R lymphocytes are infused into B10 recipients the donor cells can respond only to the H-2K or H-2D determinants, respectively. B10.A recipients of 2R or 5R lymphocytes present only H-2D or H-2K incompatibilities, respectively, to the donor cells. Using these four strains, various combinations can be made to analyze the ability of individual or combined H-2 specificities to induce in vivo lymphocyte transformation.

After lethal irradiation (850 r), recipient mice were given intravenous injections of 50×10^6 viable lymph-node lymphocytes. Donors and recipients were matched for sex. Spleens were removed daily, fixed in Carnoy's, and stained with methyl green pyronin. Three to five thousand nucleated cells per spleen were scored for mitotic figures and large pyroninophilic cells (LPC). Areas scanned were limited to regions where transformation was occurring, since the time and magnitude of transformation were the critical points.

RESULTS

The percentage of LPC and the mitotic index (MI) for days 1-6 for the isologous, H-2-incompatible, and H-2D- or H-2K-incompatible combinations in B10 recipients are plotted in Fig. 2. The isologous combination establishes baseline values of cellular activity in the absence of tissue antigen differences. The curves for the three allogeneic combinations show a marked increase in cellular activity, well above that of the isologous control. H-2 incompatibility results in an immediate increase in cellular activity, reaching a maximum at day 3, when about 30% of the nucleated cells in the regions of transformation are LPC, and on day 4, when the MI is 3.6%. The response to the H-2K incompatibility parallels the response to H-2, peaking at day 4, although the percentage of LPC seen in the transforming regions is consistently lower from days 3-6. In contrast, there is no significant cellular transformation or mitotic activity induced by H-2D incompatibility through day 3, when only a small number of LPC and mitotic figures appear in the presumptive transformation regions. Cellular activity is greatest on day 5, when the percentage of LPC is double and the MI is triple the values recorded for day 4.

Seven combinations were analyzed to compare the responses induced by different H-2D and H-2K specificities or similar segments from different strains. Curves generated from these combinations are shown in Fig. 3. Curves representing the cellular response induced by the H-2D-incompatible combinations B10.A-2R (anti-H-2.2), 2R-B10.A (anti-H-2.4,13), and B10-5R (anti-H-2.4,13,3), together with the

(F-2)

(F-3)

curve established for the basic H-2D-incompatible combination 5R-B10 (anti-H-2.2), are shown in Fig. 3a and b. All H-2D-incompatible combinations induce a similar cellular response, except for the response induced by the H-2.4,13,3 segment, which is immediate and peaks at day 4. The curves representing the cellular response induced by three different H-2K-incompatible combinations are plotted together (Fig. 3c and d) and are not significantly different.

DISCUSSION

There is ample evidence that cellular cooperation between two cell populations occurs during the antibody response to certain protein antigens.⁷ The interactions between these two populations, thymus-derived (T) and bone-marrow-derived (B) cells, and antigen involve specificity.⁸ The nature of the interaction of antigenic determinants and cells and subsequently the cell-to-cell interaction is unclear,⁹ and even less is known of the complex events which occur during the response to histocompatibility antigens. A few reports have suggested that not all determinants of the major H loci function alike,^{5,10-12} but the basis for the difference remains unclear. Data reported here show that the cellular activity induced by H-2K determinants has the same time course as an H-2 response but appears to be reduced in magnitude. H-2D determinants, nonstimulators in MLC¹¹, will induce an in vivo lymphocyte response, although it is markedly different from the one induced by H-2K determinants. The response to H-2K determinants is greatest on days 3 and 4 and declines by day 5, when the response to H-2D determinants is the greatest.

The differential response to H-2D and H-2K determinants occurs irrespective of the strain of the lymphocyte donor and recipient except in one case, B10-5R, which will be discussed later.

It has been shown that T-cell proliferation induced by oxazalone occurs in the thymus-dependent regions of lymph nodes, beginning on day 1 and reaching a peak on days 3 and 4, and that proliferation of B cells does not occur until day 5 (ref. 13). These times coincide with the timing of activation of lymphocytes by H-2K and H-2D determinants. Sprent and Miller¹⁴ showed that semiallogeneic thymus cells have a peak incorporation of [³H]thymine on day 3 after injection into irradiated recipients, and that cells that respond within 2 days to tissue antigens are cortisone-resistant T cells. Wagner et al.,¹⁵ using a one-way MLR, also showed that the effector cells in cellular immune responses to allografts are cortisone-resistant T cells. These reports suggest that the lymphocytes which respond early (days 1-3) are T cells and the cells responding on day 5 are B cells. This suggestion is supported by our observation that pretreatment of donors with cortisone does not effect the response induced by H-2K determinants but abolishes the response induced by H-2D determinants.¹⁶ If different portions of the H-2 complex stimulate different lymphocyte populations, the sequence of events in the immune response to tissue antigens may be interpreted in the context of the hapten-carrier systems, where T cells are carrier-specific and the antibody-producing cells are hapten-specific.^{7,17} Thus, we could consider that the serologically defined H-2 specificities act as haptens, turning on B cells to produce the specific antibodies that have been used to identify the H-2 specificities

as we know them. A heretofore unidentified thymic dependent determinant(s) would provide the carrier function that turns on T cells to act as "killer cells" in cell-mediated immunity and to act as "helper cells" in inducing or enhancing the B-cell response. The appearance of the late response of B cells to H-2D determinants is consistent with evidence that the hapten response is dose-dependent⁹ and the carrier effect enhances a weak or minimal response. Lilly¹⁸ has shown that H-2.2 antigen alone does not induce detectable antibody levels.

It is of interest that the one H-2D incompatibility which induces early (T-cell) transformation, B10→5R, is the reciprocal of the combination in which the H-2D-induced late (B-cell) transformation was first noted, 5R→B10. In both combinations the lymphocytes are reacting to allelic segments of the H-2 locus. B10 has a determinant that can induce early transformation, as seen in the combination 2R→B10, but it apparently resides at a position to the right of H-2.5 in the locus (Fig. 1). However, the segment of H-2^a that 5R obtained in the crossover contains a determinant that will induce early transformation of B10 cells. Therefore, the thymic dependent determinant(s) does not appear to occupy an allelic position(s) in all H-2 loci. The one serologically identifiable difference on 5R cells determined by this segment is H-2.3. Shreffler⁶ has suggested that H-2.3 evolved from the H-2K region through a postulated inverted duplication centering around Ss-Slp, and it is tempting to speculate that H-2.3 is the factor; but it is equally likely that the determinant is a nonserologically defined structure whose existence is signaled by its unique capacity to turn on T cells. Benaceraff et al.¹⁹ have suggested that the Ir-1 locus

may be a thymus receptor site, but in this instance the Ir-1 alleles are identical.⁶

Although the Slp alleles differ in this combination, T-cell activation occurs in combinations where the Slp alleles are in common.

The data presented here show that the antigenic sites determined by different portions of the H-2 locus function differently in the immune response. Determinants of H-2D segments of the H-2 locus induce lymphocytes to transform later than determinant(s) of the H-2K segments. Factors such as the time of the response and the sensitivity of the H-2D-induced response to cortisone suggest that a determinant(s) located in the H-2K region stimulates T cells and H-2D segments lacking this determinant stimulate B cells. It is further suggested that the total product of the H-2 locus may react in the immune response much like the synthetic hapten-carrier antigens in inducing a synergistic reaction between two populations of lymphocytes, resulting in an immune response characterized by cell-mediated immunity and antibody production.

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FIGURE LEGENDS

Fig. 1. — Simplified recombination maps showing linear order of specificities pertinent to this study. Vertical bars designate subdivisions defined by recombination, arrows the position of recombination producing 2R and 5R alleles, and numbers serologically defined H-2 specificities.

Fig. 2. — Cellular activity in spleens of lethally irradiated mice receiving isologous (B10→B10), H-2-incompatible (B10.A→B10), H-2D-incompatible (5R→B10), and H-2K-incompatible (2R→B10) lymphocytes. Points represent arithmetic means ($\pm$ S.E.) of the percentages of LPC and the MI from four mice.

Fig. 3. — Cellular activity in spleens of lethally irradiated mice receiving (a, b) H-2D-incompatible lymphocytes, 5R→B10 (anti-H-2.2), B10.A→2R (anti-H-2.2), 2R→B10.A (anti-H-2.4,13), and B10→5R (anti-H-2.4,13,3); (c, d) H-2K-incompatible lymphocytes, 2R→B10 (anti-H-2.33), B10.A→5R (anti-H-2.33), and 5R→B10.A (anti-H-2.1,8,11,23,25). The broken lines represent the basic H-2D- and H-2K-incompatible response taken from Fig. 2 and are shown for comparison.

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Fig. 1

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Fig. 2

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Fig. 3