Brief Definitive Report

EFFECTS OF ANTI-Ia SERA ON MITOGENIC RESPONSES III. Mapping the Genes Controlling the Expression of Ia Determinants on Concanavalin A-Reactive Cells to the *I-J* Subregion of the *H-2* Gene Complex*

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The *I* (immune response) region of the mouse major histocompatibility complex codes for a series of lymphocyte membrane alloantigens (Ia), as well as many immunologically related functions, including immune response genes, graft versus host reactivity, and mixed lymphocyte reactivity (1). Although there has been some controversy concerning the expression of Ia determinants on T cells (2–6), there is now agreement that Ia antigens are expressed on at least a subset of T cells, as well as most B cells (7–12).

We have previously shown that the simple addition of antisera directed at *I*-region determinants to either whole spleen or a splenic B-cell population will partially inhibit the mitogenic response to lipopolysaccharide (LPS). Since Ia is expressed on most B cells, it was not surprising that treatment with anti-Ia and complement can remove the ability of spleen cells to respond to LPS by increased thymidine incorporation (13). In contrast, simple treatment with anti-Ia antibodies alone, without complement, does not affect either the Concanavalin A (Con A) or phytohemagglutinin (PHA) mitogenic responses of spleen cell cultures. However, removal of the Ia-positive cells from T-cell preparations with anti-Ia plus rabbit complement removes Con A, but not PHA reactivity of these populations (11).

Okumura et al. have reported that Ia is expressed on suppressor T cells (8), and further, Murphy et al. have shown that these determinants map in the interval between *I-B* and *I-C* regions in a new region designated *I-J* (9). The relationship between the Con A-reactive T cells and suppressor T cells is not yet clear, but it is known that Con A stimulation can induce suppressor activity in spleen cell cultures (14).

We have shown (15) that only a small portion of T cells directly respond to Con A. This Ia-positive population can promote the mitogenic response of other Ia-negative T cells to Con A, hence the name, promotor T cells. The relationship of the Ia-positive Con A-responsive T cell to other previously defined T-cell subclasses described by other cellular alloantigens, particularly Ly or Fc determinants, is not yet clear (16–18).

Materials and Methods

Mice. All mice were raised in the breeding colonies at the University of Southern California or University of Michigan.

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Antisera. Anti-Ia sera was produced as previously described. Details of the donor-recipient combinations are given in Table I. One batch of A.TL anti-A.TH was generously provided by Dr. Donal Murphy.

Antiserum Treatments. Cell suspensions were treated with antiserum, or normal mouse serum followed by complement. In brief, cells were suspended at a concentration of 10^7 cells/ml in a dilution of antiserum in RPMI 1640, incubated at 37°C, centrifuged, resuspended in an equal volume of titrated agarose-EDTA or mouse spleen cell-absorbed rabbit complement, incubated 30 min longer, washed three times in H-3 medium without serum, and counted (11). Dye exclusion microcytotoxicity tests and absorptions were performed as previously described (5).

T-Cell Purification. T cells were purified by filtration on nylon wool columns (19). Less than 5% of these cell populations are Ig^+ by immunofluorescence.

Culture Conditions. Mitogen stimulations were carried out in 96 well Microtest II plates (Falcon Plastics, Oxnard, Calif.) in serum-free H-3 medium as previously described (11). Cultures of 5×10^5 T cells were labeled during the final 4 or 18 h with [^{3}H]thymidine (2 Ci/mmol; 2 μCi/well) of the 72-h culture period. Cultures were harvested using a multiple sample harvestor (Otto Hiller Co., Madison, Wis.) and counted in a liquid scintillation counter.

Results and Discussion

Mapping the Ia Determinants Expressed on Promotor T Cells. To map the determinants expressed on the Con A-reactive cells to a specific portion of the *H-2* complex, we used a series of antisera designed to produce antibodies against restricted portions of the *I* region of the mouse *H-2* complex. We tested a number of these antisera for their ability to remove promotor T-cell activity (Table I). Serum A.TH anti-A.TL removed the reactivity of B10.D2 T cells to Con A. This suggested that the cytotoxic reaction of this serum with B10.D2, which defines specificity Ia.7 (1), mapped the promotor activity to the *I-C* region. However, another serum [129 × B10.A(4R)]F_1 anti-B10.A(2R) also directed at I-C determinants (Ia.6 and Ia.7) failed to affect the Con A response B10.A(3R) of T cells, although the serum does react with 3R lymph node and spleen cells in the microcytotoxic test. This suggested that either the Con A promotor reactivity mapped to the left of the B10.A(3R) crossover and failed to react in B10.A(3R), since it carried a portion of the $H\text{-}2^b$ haplotype in this region, or that the [129 × B10.A(4R)]F_1 anti-B10.A(2R) merely failed to contain the relevant antibody, although it might have. Later we will present evidence that the first explanation is correct. Another antiserum (129 × A.TH)F_1 anti-A.TL when tested on B10.A(4R) targets also fails to affect the Con A response, even though it is cytotoxic for B10.A(4R) lymph node and spleen cells in cytotoxic tests. These data map the reactivity to the *D* side of the B10.A(4R) recombinant. Although we realize the caveats implied by negative results, both of these observations are consistent with the assignment of the promotor specificity to the *I-J* subregion, between the *I-B* and *I-C* regions. A different antiserum (A.BY × B10.HTT)F_1, anti-A.TL, when used to treat B10.A(4R) T cells, gives similar results. This antiserum does contain anti-*I-J* reactivity since it can remove the Con A response of B10.K cells.

In a further attempt to determine if any promotor cell specificities mapped in the *I-A* region, we tested antiserum (A × B10.D2)F_1 anti-B10.A(5R) on a cross-reactive target cell, B10.S. This reaction defines specificity Ia.9 known to map in the *I-A* region. Although this antiserum contains antibodies reactive with $H\text{-}2K^b$ specificities, no *H-2* cross-reactions are known to occur with this serum using B10.S target cells. This antiserum has no effect on the Con A response of B10.S T cells after treatment with serum and complement. We also tested this

TABLE I
Mapping the Ia Specificity on the Promoter Cell

Antiserum		Target cell	*I* subregion tested	[^{3}H]thymidine ± SD
Recipient	Donor			
				cpm
A.TH	anti-A.TL	B10.D2	*I-C?*	15,873 ± 3,790
None				159,040 ± 1,463
NMS*				60,425 ± 7,377
[129 × B10.A(4R)]F_1	anti-B10.A(2R)	B10.A(3R)	*I-E,I-C,S*	61,020 ± 6,903
None				75,554 ± 4,879
NMS				91,688 ± 1,263
[129 × A.TH]F_1	anti-A.TL	B10.A(4R)	*I-A*	148,854 ± 3,012
None				151,800 ± 3,554
NMS				170,210 ± 11,095
[A.BY × B10.HTT]F_1	anti-A.TL	B10.A(4R)	*I-A*	72,624 ± 884
None				63,522 ± 901
NMS				52,666 ± 3,239
[A × B10.D2]F_1	anti-B10.A(5R)	B10.S	*I-A?*	100,424 ± 43,644
None				156,055 ± 18,487
NMS				93,654 ± 39,950
[A × B10.D2]F_1	anti-5R absorbed with EL-4	B10	*I-A*	106,875 ± 25,786
None				105,187 ± 27,771
NMS				135,462 ± 17,271
[A.TH × B10.HTT]F_1	anti-A.TL	B10.A(9R)	*I-J*	3,215 ± 1,157‡
NMS				15,543 ± 4,492‡
[A.TH × B10.HTT]F_1	anti-A.TL	B10.A(5R)	*I-J*	3,695 ± 620‡
NMS				27,024 ± 6,000‡
[A.TH × B10.HTT]F_1	anti-A.TL	B10.A(3R)		11,090 ± 1,660‡
NMS				12,950 ± 4,678‡
A.TL	anti-A.TH	B10.HTT	*I-A,I-B,I-J*	127,364 ± 4,836
None				123,914 ± 11,275
NMS				121,130 ± 3,841

* NMS, normal mouse serum.
‡ 4-h label.

serum on the homologous B10 T-cell target. David et al. have reported that the T-cell reactivity of blasts is considerably weaker when tested on cross-reactive target cells than homologous target cells (20). To test the possibility that we could detect the relevant antibody using an *H-2*^{b} target, it was necessary to first absorb this serum with EL-4 tumor cells to remove the anti-H-2K^b antibodies. EL-4, although a *H-2*^{b} thymic leukemia, does not appear to express any Ia antigens, but does express *H-2K*^{b} specificities. After this absorption, the serum remains cytotoxic for B10 lymph node cell targets, but not EL-4 cells. Treatment with this absorbed serum and complement had no effect on the Con A response of B10 T cells. While again a negative result, this provides further evidence that *I-A* subregion specificities such as Ia.9 are not expressed on promotor T cells for the Con A response.

The critical recombinants for mapping the promoter response are the B10.HTT and B10.S(9R) pair and the B10.A(3R) and B10.A(5R) pair. We produced an antiserum (A.TH × B10.HTT)F_1 anti-A.TL which should contain antibodies against the *I-A*^{k}, *I-B*^{k}, and *I-J*^{k} regions, but not the *I-E*^{k} or *I-C*^{k}

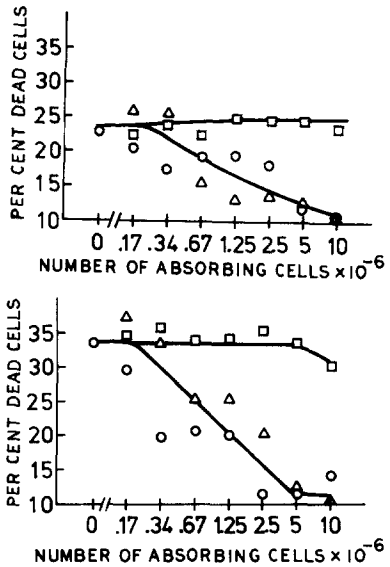

FIG. 1. In vitro absorption of serum (A.TH × B10.HTT)F_1 anti-A.TL by B10.A(3R) and B10.A(5R). (Top) Serum absorbed with B10.A(3R) (○—○), B10.A(5R) (△—△), or B10.HTT (□—□) spleen cells and tested against B10.A(3R) lymph node cell targets. (Bottom) Same absorbed sera tested against B10.A(5R) lymph node cell targets.

regions. When this serum is tested B10.S(9R) T cells, the Con A response is destroyed. Similarly, when tested on B10.A(3R) and B10.A(5R), there is no effect on the B10.A(3R) response, but the B10.A(5R) mitogenic response is greatly inhibited even though B10.A(3R) and B10.A(5R) share $I\text{-}A^b$, $I\text{-}B^b$, $I\text{-}E^k$, and $I\text{-}C^d$ regions. These combinations together map the response to the interval defined by the B10.A(3R)–B10.A(5R) and B10.S(9R)–B10.HTT recombinant pairs. Only one antiserum tested, a single batch of A.TL anti-A.TH tested on B10.HTT cells, failed to show anti-promotor activity where it is expected on the basis of $I\text{-}J$-region differences. It is, of course, possible that while this antibody might have been present, it was merely absent from the single bleeding tested. As we noted earlier, other bleedings of A.TL anti-A.TH do contain anti-promotor activity (11), but none of these have been tested on B10.HTT. It is worthwhile to note that serum (A.TH × B10.HTT)F_1 anti-A.TL reveals no detectable cytotoxicity when tested with B10.S(9R) lymph node or spleen cells, although it does kill both B10.D2 and B10.A spleen or lymph node cells. It also reacts equally with B10.A(3R) and B10.A(5R) cells in cytotoxic tests. This cytotoxic reactivity can be explained by either anti-Ia.8 and/or anti-Ia.15 and/or Ia.22. However, B10.A(3R) and B10.A(5R) can remove cytotoxic activity from this serum reciprocally (Fig. 1). Thus the specificity in the $I\text{-}J$ region seems to be different from other previously defined Ia specificities.

One point which bears further discussion is the derivation of B10.A(3R)–B10.A(5R) and B10.A(9R)–B10.HTT pairs in the $I\text{-}J$ subregion. We have some evidence the $I\text{-}J^k$ and $I\text{-}J^d$ products can cross-react. For example, A.TH anti-A.TL removes Con A reactivity of B10.D2 targets. This is a cross-reaction of $I\text{-}J^d$ with antibodies directed against $I\text{-}J^k$. It is simplest to describe the results with

B10.HTT–B10.S(9R) by ascribing the *I-J* region of B10.HTT to $H\text{-}2^s$ and the *I-C* region to $H\text{-}2^k$. Since B10.S(9R) is derived from $H\text{-}2^{a/s}$ recombination rather than $H\text{-}2^{t1/s}$ as in B10.HTT, the *I-J* region of B10.S(9R) might come from either $H\text{-}2^k$ or $H\text{-}2^d$, but just as in the B10.A(3R)–B10.A(5R) pair, the simplest explanation is that B10.A(3R) derives its *I-J* region from $H\text{-}2^b$, while B10.A(5R) derives its *I-J* region from $H\text{-}2^k$. This is the most economical explanation, since the *I-E* region of B10.S(9R), B10.HTT, B10.A(3R), and B10.A(5R) are all apparently identical and derived from the $H\text{-}2^k$ haplotype (David, C. S. personal communication).

In these experiments we have been unable to distinguish between $I\text{-}J^d$ and $I\text{-}J^k$, although this does not rule out that later such a distinction might be made with other antisera. In fact, preliminary data suggest that $(C3H.Q \times B10.D2)F_1$ anti-AQR has such an antibody which can distinguish between $I\text{-}J^d$ and $I\text{-}J^k$.

It is surprising to note that unlike the effects of Ia sera we have reported on secondary Mitchell-Dutton cultures, where simple treatment of spleen cells with antisera directed at either the *I-A* or *I-C* subregion is sufficient to disrupt the secondary IgG response (21), the promotor activity is localized to only a single subregion of the *H-2* complex. The results reported there are similar to those of Murphy et al. which showed that the Ia determinants expressed on the allotype suppressor T cell map to a single *I* subregion (9). Thus it may be that the Ia determinants are functionally segregated in specific regions rather than interspersed in the *H-2* gene complex as our previous data had suggested.

Summary

We have shown that the Ia determinants expressed on nylon wool-purified T lymphocytes reactive to concanavalin A (Con A) in serum-free media are coded in a single *I* subregion of the *H-2* gene complex. This region, *I-J*, is defined by two pairs of intra-*H-2* recombinant haplotypes: $H\text{-}2^{t3}$, $H\text{-}2^{t4}$ and $H\text{-}2^{i3}$, $H\text{-}2^{i5}$, carried by B10.HTT, B10.S(9R), B10.A(3R), and B10.A(5R), respectively. No activity against Con A-reactive T cells has been detected in any antiserum that was produced in strain combinations which shared a common *I-J* region. This suggests that Ia antigens expressed on Con A-reactive T cells are restricted to the *I-J* subregion.

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