THE DIFFERENTIAL DESTRUCTIVE AND ENHANCING EFFECTS OF ANTI-H-2K, H-2D, AND ANTI-Ia ANTISERA ON MURINE SKIN ALLOGRAFTS*

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In the mouse the H-2 complex is divided into a number of regions consisting of K, I, S, G, and D, (1). Of these, the I region has proven to be of particular interest as it contains genes which effect a number of diverse biological phenomena. Included in these are the histocompatibility (H) I region genes and it has been of interest to compare the different K, I, and D (H) genes. Each of these regions contain one or more genes which code for strong histocompatibility effects (H-2K and H-2I), or weaker effects (H-2D, H-2IC) (2, 3). Comparisons of the serologically detected H-2K and H-2D specificities with the I region Ia specificities have demonstrated these specificities to be clearly different, not only in their tissue distribution and function (1), but particularly with regard to the production of enhancement with antisemur. As shown initially by absorption (4) and more recently, with the appropriate strain combinations (5, 6), anti-Ia sera can lead to skin graft enhancement for both K and I region differences, whereas anti-H-2K sera, lacking an Ia component, does not do so. These results therefore led to the question of whether anti-H-2K or D sera, under appropriate conditions (7, 8), were graft destructive, and indeed, as shown herein, this is the case, whereas anti-Ia sera were shown not to be so.

Materials and Methods

Mice. The mice used are listed in the Tables. BALB/c-nu/nu mice were obtained from Dr. M. Holmes, Walter & Eliza Hall Institute, Melbourne and CBA/H-nu/nu from Dr. J. B. Smith, John Curtin School of Medical Research, Australian National University, Canberra.

Antisera and Serology. Alloantisera and anti-lymphocyte sera (ALS) were produced as previously described (7, 9) and were tested in a two stage cytotoxic assay (9). The alloantisera were raised to detect either H-2K, D, or Ia specificities (Tables I and II, footnotes). Alternatively, sera were made K, D, or I region specific by absorbing with either normal lymphoid cells (see Tables) or with tumor cells: EL4 (an H-2b leukemia which is H-2K+, H-2D+, Iα–) or MOPC-315 (an H-2b plasmacytoma which is H-2K+, H-2D+, Iα+). In each case the tissues were obtained as a cell suspension, washed twice in medium, and used to absorb antisemur, (1 vol of cells: 3 vol antisemur at a dilution of 1:2) for 30 min at 4°C and 30 min at room temperature. Rabbit serum as an in vivo source of complement was obtained by bleeding rabbits on the day of the study and the sera rapidly separated and used.

Protocol for Studying Antibody-Mediated Graft Rejection in Vivo. The design of these experiments was based on the observation that in mice whose cellular immune mechanisms are suppressed, antibody-mediated graft destruction can be observed provided that a potent source of complement is used (7, 8). In the mice suppressed with ALS, skin grafts were performed and the recipients received 0.25 ml of ALS on days 1, 2, and 4 with respect to grafting. As expected, these

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Table I

Antibody-Mediated Graft Destruction Experiments in Mice Suppressed with ALS

| Antiserum + RC | Specificity detected | Survival time of graft | Time from serum injection to rejection |
|---------------|----------------------|------------------------|---------------------------------------|
| (a) B10.A(8R) Donor* |                      |                        |                                       |
| 1. * RC       | -                    | >35 × 6                | -                                    |
| 2. AS630      | H-2K^a, Ia^b         | >35 × 6                | >25                                   |
| 3. AS630 + RC | H-2K^a, Ia^b         | 11, 11, 12, 11, 13    | 2-3                                   |
| 5. AS630 absorbed EL4 + RC | In.9          | >35 × 6                | >25                                   |
| 6. AS630 absorbed | H-2K^b         | >35 × 24               | >25(x2)                               |
| B10.8(Rb) + RC | H-2K^c           |                        |                                       |
| (b) B10.A(2R) Donor |                      |                        |                                       |
| 7. AS630 + RC | H-2D^d             | 11, 11, 12             | 2-3 (x3)                              |
| 8. RC         | -                   | >35 × 4                | >25                                   |

* In all experiments (B10.D2 × A)F1 male mice were suppressed with ALS 0.25 ml on days 0, 2, and 4. Groups of four to six mice received a skin graft on day 0 and day 9, alloantiserum and RC (see text). The alloantiserum (AS630) was made as (B10.D2 × A/F1, anti-C57BL/6 and reacted with K^b, P, and D^b specificities (C57BL/6 spleen; titer 1/4,000); I^a and D^b (EIA: 1/4,000); In.9 (B10.S(gR): 1/128); K^b1 (B10.A(5R): 1/2,500); H.21T (B10.A(2R): 1/128); H-2K^b (serum after absorbing with B10.S(gR): B10.A(SR): 1/2,000).

† These grafts showed early changes of rejection but subsequently recovered.

Results

The models chosen for studying the effects of graft rejection due to antibody and rabbit complement (RC), in the absence of cellular immunity, appeared to be satisfactory, as neither ALS treated, nor nude mice rejected their grafts within the period of observation (25–35 days) (Table I, group 1; Table II groups 1, 7, and 12). Presumably the nude mice would have retained their grafts permanently, whereas the ALS-treated mice eventually rejected their grafts. Suppressed mice given fresh rabbit serum as a source of complement also retained their grafts (Table I, groups 2 and 8, Table II, groups 2, 8, 13, and 20). In addition, in a limited experiment (Table I, group 3) alloantibody alone did not cause graft rejection. Mice treated with antiserum plus RC were able to reject their grafts (Table I, groups 4, and 7, Table II, groups 3, 9, 10, 14, 15, 17, and 18) in acute fashion, i.e. within 2–5 days of receiving antiserum and complement. All mice thus treated showed evidence of graft rejection but several grafts recovered and avoided total destruction (Table I, groups 6 and 7, Table II, group 18). The results and the observations on the mode of graft rejection described herein conform entirely to the original descriptions of this phenomena (7, 8). Attempts to dissect the phenomena of antibody-mediated graft rejection in terms of the specificity of the antisera were then made. Most of the antisera used contained antibodies to H-2K or H-2D alloantigens and in addition, one or more known Ia specificities. Sera were therefore made specifically reactive with either H-2K, H-2D, or with Ia specificities either by absorption or by producing antisera in combinations differing only in that particular region.
### Table II
Antibody-Mediated Graft Destruction in BALB/c and CBA-nu/nu Mice Receiving Different Skin Grafts

| Time from serum injection to rejection | Antiserum + RC* | Antiserum number | Survival time of grafts |
|---------------------------------------|-----------------|-----------------|------------------------|
| days days | days |

(a) B10.A(5R) Donor, BALB/c-nude recipient

1. – – >33 –
2. RC – – >33 –
3. H-2K\(^\text{b}\), Ia\(^\text{a}\) + RC AS630 11 × 6 2
4. Ia.3 + RC AS608 >33 × 6 >33
5. H-2K\(^\text{b}\), Ia\(^\text{b}\) + RC AS306 11 × 4, 12 × 2 2-3
6. Ia\(^\text{a}\) (Ia.3) + RC AS06 absorbed EL4 >33 × 6 >25

(b) CBA Donor, BALB/c-nude recipient

7. – – >25 –
8. RC – – >25 –
9. H-2D\(^\text{a}\) + RC AS292 12, 12, 13, 14>25 × 2 3-5
10. H-2K\(^\text{a}\), Ia\(^\text{a}\) + RC D23 12, 12, 13, 13 >25 × 1 3-4
11. Ia\(^\text{a}\) + RC AS608 >25 >15

(c) C57BL/6 Donor, BALB/c-nude recipient

12. – – >25 –
13. RC – – >25 –
14. H-2K\(^\text{a}\), Ia\(^\text{a}\), H-2D\(^\text{a}\) + RC AS630 >25 2
15. H-2K\(^\text{a}\), H-2D\(^\text{a}\) + RC B10.S(9R) AS630 absorbed EL4 11 × 6 2
16. Ia\(^\text{a}\) (Ia.9) + RC AS608 absorbed EL4 >25 >16
17. H-2K\(^\text{a}\), P, H-2D\(^\text{a}\) + RC AS630 absorbed MOPC-315 11 × 5 2

(d) C57BL/6 Donor, CBA-nude recipient

18. H-2D\(^\text{a}\) + RC AS630 12, 12, 13, 14 3, 3, 4, 5
19. Ia\(^\text{a}\) (Ia.8) + RC AS742 >35 × 6 >25 × 6
20. RC – – >35 >26

* Groups of five to six male nude mice were used as recipients. Sera were produced as (a) AS630—see Table I. (b) AS306 (anti-K\(^\text{b}\)) (B10.D2 × A)F, anti-B10.A(5R): activity (K\(^\text{b}\)) C57BL/6 and B10.A(5R): 1/1,000; (K\(^\text{d}\)): EL4 1/500; (P): B10.S(9R): 1/2; (c) AS608 absorbing EL4 (anti-P + Ia): B10.A(5R): 0/64. (d) AS808: A.TH anti-A.TL: B10.A(5R) (Ia.7, 7, 15); 1/150, CBA-I\(^\text{a}\) : 1/1,000. (e) AS303 (IT) (B10.D2 × A)F, anti-B10.A(5R); 1/400 on C57BL/6. (f) AS742: (Ia.8) (B10.A × A)F, anti-B10.A × AIF; anti-B10.D2: 1/128 on C57BL/6.

The use of such specific antiserum clearly pointed to the graft destructive effects of anti-H-2K and H-2D antisera, and the lack of such effects with anti-Ia antisera. For example, graft destruction was produced by AS630 + RC (Table I, group 4) containing anti-H-2K\(^\text{b}\) and Ia specificities. When the H-2K activity was removed by absorbing with EL4 (Table I, group 5), the destructive capacity of the antiserum was lost, whereas after removal of the Ia antibody by absorption with B10.S(9R) (Table I, group 6), the antiserum was still fully destructive. A similar but weaker reaction was obtained with this serum against the H-2D\(^\text{a}\) specificities (Table I, groups 7 and 8), i.e. in this combination, in ALS-suppressed mice, anti-H-2K or H-2D antibodies were destructive, anti-Ia antibodies were not. It should be noted that the absorption with EL4 tumor was specific for the H-2K, and D specificities under study, as absorption with another tumor, MOPC-315 (Table II, group 17) was without effect on the
Further examples of the differential effects of H-2K, D, and Ia antisera were shown in Table II with athymic (nude) recipients. In these studies, anti-H-2K\(b\) (Table II, groups 3, 5, 14, 15, and 17), anti-H2K\(k\) (Table II, group 10) and anti-H2D\(k\) (Table II, groups 9 and 18) were destructive whether Ia specificities were present or not. By contrast anti-Ia\(b\) (Ia.3, 8, 9) or anti-Ia\(k\) (Ia.1, 2, 3, 19, 22) (Table II, groups 6, 11, 16, and 19) antisera had no effect, acutely, on the fate of the grafts. One problem encountered with the H-2\(b\) reagents could be the low titer of anti-Ia.9 in the serum, i.e. it could be argued that the lack of destructive effect of anti-Ia was due to too little antibody. This possibility was considered to be unlikely in view of the inability of anti-Ia\(k\) sera, (Table II, group 11) with a cytotoxic titer 1/2,000, to cause graft destruction when used alone. Clearly, H-2K and H-2D antibodies can destroy grafts, anti-Ia antibodies cannot do so.

**Discussion**

The in vivo reactions of anti-H-2 sera can now be regarded as being complex and made up of the different reactions of antisera to the different components, i.e. anti-H-2K, D, and anti-Ia. These different reactions were most clearly observed in studies of passive enhancement wherein anti-H-2 sera with the Ia reaction removed by absorption were unable to enhance, whereas those with anti-Ia serum retained, e.g. after absorption with erythrocytes or with EL4, could still produce enhancement (4) but not destruction (4, 10). Using special combinations, we were able to confirm these findings and demonstrate that anti-Ia antisera could suppress or delay graft rejection not only with I region antigenic differences, but also with \(K/D\) differences (5, 6) and it is not unlikely that anti-Ia antiserum delays induction of immunity by removal of Ia antigens which appear to selectively stimulate helper T cells (11).

It should be noted that these observations on enhancement apply, not only for skin grafts in mice, but also for other tissues in other species (4), and this may include studies in monkeys (12) and man (13-16). Why there are differences in the susceptibility of grafts to destruction by anti-Ia antibodies + RC and anti-H-2K/D antibodies is not clear. Anti-Ia antibody could conceivably fail to cause graft rejection for the following reasons: (a) absence or low density of Ia antigens on skin tissues; (b) rapid turnover of antigens; (c) immunoglobulin class of anti-Ia antibodies leading to poor fixing of complement; or (d) failure of antibody to reach the graft. The question of the presence of Ia antigens in epidermal and endothelial cells, and their density, has not been finally settled, but Ia antigens have been directly demonstrated on epithelial cells, by cytotoxicity (17), so that, presumably the density of receptors is such that complement can be fixed - at least in vitro (17). In addition, anti-Ia antibodies of high titer are formed after skin grafting, indicating the presence of Ia antigens within the graft (2, 6). Ia antigens could conceivably have a very rapid in vivo turnover and generate complexes so rapidly that insufficient antibody of this specificity remains fixed to the cell surface to cause lysis. This is a possibility, especially in view of our findings on the secretion of Ia antigens into the serum by stimulated lymphocytes (18), but in the absence of direct measurement of secretion rates this possibility must remain theoretical. It is also unlikely that noncomplement fixing antibody blocks or inhibits graft destruction as this is not observed in vitro. However, there may be technical problems in antibody reaching the graft, including the immunoglobulin class of the antibody. Also, if Ia specificities are carbohydrate in nature (19), then the microheterogeneity of carbohydrate structures
could lead to the in vivo absorption of antibody by the host, so that little or none is
available to bind directly to the graft. At present, none of these explanations would
appear to be acceptable in the absence of any experimental data and the observations,
although valid, remain unexplained. However, whatever the mode of action of Ia
antisera in prolonging (i.e. enhancing) skin grafts rather than leading to their destruc-
tion, as do anti-H-2K/D antisera, the observations could be considered to be of practical
importance for tissue allografting in man. The HLA-A, B loci are considered to have a
homology with the H-2K, D loci in the mouse. In man, antisera to HLA-A and B
specificities lead to rapid, "hyperacute" rejection of renal allografts, marrow, and
platelet grafts (16, 20). It is now apparent that the recently described B-cell alloantisera
in man which map to the HLA-A and D loci and which appear to be completely
analogous to the Ia system in the mouse, are nondestructive for renal allografts (13-15).
If this is so, then the homology may extend further, and these antibodies could be the
specific antibodies required to produce allograft enhancement in man.

Summary
Nude (athymic) or anti-lymphocyte serum-treated mice have absent or
delayed graft rejection due to impaired T-cell responses. Nonetheless, these
mice can reject skin grafts, acutely, when treated with anti-H-2 antibody and
additional complement. Resolution of the components in the H-2 antisera, by
either absorption or by selective production of antisera of restricted specificity,
has demonstrated that anti-H-2K or H-2D antisera are graft destructive, and
as shown elsewhere, are nonenhancing. By contrast, anti-Ia sera are not
capable of causing allograft destruction even when used in extremely high
doses and were previously noted to be enhancing. The mechanism of such
differential effects is not apparent, but the findings are clearly of importance to
transplantation in man, where sera reacting specifically with B cells may also
be enhancing and nondestructive.

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