THE ROLE OF H-2 AND Ia ANTIGENS IN GRAFT-VERSUS-HOST REACTIONS (GVHR)

Presence of Host Alloantigens on Donor Cells after GVHR and Suppression of GVHR with an Anti-Ia Antiserum Against Host Ia Antigens*

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Graft-versus-host responses (GVHR) occur when immunocompetent lymphoid cells are transferred from a donor to an allogeneic host (1). Donor lymphocytes recognize alloantigens on host cell membranes and are stimulated to proliferate. If the host has a reduced immune competency (neonatal mouse or immunosuppressed recipient), then donor cells can invade and damage host tissues causing a runting syndrome, termed graft-versus-host disease (GVHD). This condition is manifested by weight and hair loss and diarrhea. In severe cases, it results in death of the recipient. Clinically, GVHD frequently follows bone marrow transplantation (2).

The GVHR can be divided into a proliferative phase and an effector phase which correspond to the in vitro mixed lymphocyte reaction (MLR) and cell-mediated lympholysis (CML) reaction, respectively (3). The proliferative phase of the GVHR can be quantitated by measuring splenomegaly in a spleen weight assay (1), or by a lymph node weight assay (4). The effector phase of the GVHR (manifested as GVHD) may be quantitated by recording the survival time of the recipient and by observing the recipients for signs of GVHD (3).

Previous studies of the GVHR have demonstrated that the main stimulus for the proliferative phase of the GVHR is provided by an incompatibility in the I-A subregion of the mouse H-2 major histocompatibility complex (MHC) (3, 5–7). K- or D-region differences induce much weaker GVHR, whereas I-C-subregion differences require the preimmunization of donors to produce significant GVHR (3, 5, 6). In GVHD, K, I-A, or D differences induce about the same degree of mortality (3), although I-C differences require the preimmunization of donors to cause mortality (5, 6). The I-B and I-J subregions have not been shown to induce GVHR (5). Minor H loci can induce GVHR provided donor cells are presensitized and injected intravenously (i.v.) in high doses (7).

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Abbreviations used in this paper: AEF, allogeneic effect factor; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; I, immune-response region; Ia, I-region associated antigen; GVHD, graft-versus-host disease; GVHR, graft-versus-host response; MLR, mixed lymphocyte culture reaction; NMS, normal mouse serum; PBS, phosphate-buffered saline; TRITC, tetramethyl rhodamine isothiocyanate.
Table I

H-2 Haplotype Origin of Strains Used

| Strain         | Haplotype | Region* | S | G | D |
|----------------|-----------|---------|---|---|---|
|                |           | K       | A | B | J | E | C |
| B10.BR         |            | k       | k | k | k | k | k |
| B10.S          |            | s       | s | s | s | s | s |
| B10            |            | b       | b | b | b | b | b |
| A.TH, B10.S(7R)| i2        | s       | s | s | s | s | s |
| A.TL           | i1        | s       | k | k | k | k | k |
| B10.HTT        | i3        | s       | s | s | k | k | k |
| B10.A(3R)      | i3        | b       | b | b | k | k | k |
| B10.A(5R)      | i5        | b       | b | b | k | k | k |
| B10.AQR        | y1        | q       | k | k | k | d | d |
| B10.T(6R)      | y2        | q       | q | q | q | q | q |

* Haplotype of origin according to references (44, 45).

The GVHR represents the first phase in our procedure used to generate a soluble, lymphocyte-derived, allogeneic effect factor (AEF) (8). An analysis of the activity of an H-2-restricted AEF has indicated that such an AEF, produced across an entire H-2 region disparity, contains Ia antigens determined by the I-A subregion of the stimulator haplotype (8). This AEF will help B cells of the stimulator but not responder haplotype, and will only help B cells of other strains which express the same I-A determinants as the stimulator cells. This H-2 restriction of the activity of AEF has now been observed for several AEFs produced across various I-subregion differences (9). However, the mechanism which controls this H-2 restriction phenomenon is not yet understood.

Recently, Nagy et al. (10, 11) have reported that different subpopulations of responder T-cell blasts can during an MLR absorb either irradiated stimulator cell derived H-2 or Ia antigens. In an attempt to understand the basis for the H-2 restriction of AEF activity described above, we examined the ability of responder T-cell blasts to acquire H-2 and Ia antigens of the stimulator haplotype during a GVHR.

In this study, we demonstrate that donor T cells which mediate a GVHR do not express Ia antigens determined by the donor haplotype but do acquire Ia antigens determined by the recipient haplotype. In addition, we show that injection of recipients during the proliferative phase of the GVHR with an antiserum directed against recipient Ia antigens can markedly suppress the GVHR. Based on these data, a model is presented which attempts to explain the role of Ia antigens in the genetic control of allogeneic lymphocyte interactions.

Materials and Methods

Mice. Inbred strains of mice were raised in our mouse colony at University of Toronto. The B10.AQR and B10.T(6R) mice were a generous gift from Dr. R. A. Phillips, Department of Medical Biophysics, University of Toronto. The H-2 haplotypes of the strains used in this study are listed in Table I.

Antisera. The hyperimmune antisera used in this study and the respective H-2 regions immunized against are as follows: A.TH anti-A.TL (anti-I^a, S^a, G^a); A.TL anti-A.TH (anti-I^a, S^a, G^a); (A.TH × B10.HTTF)F1 anti-A.TL (anti-I^a, I^B^a, I^J^a); B10.S(7R) anti-B10.HTT
The sera were raised by hyperimmunization of recipient mice with donor spleen and lymph node lymphocytes as described by Okumura et al. (12). The IgG fractions of these antisera were prepared by ammonium sulphate precipitation followed by ion-exchange chromatography on QAE Sephadex A-50 (Pharmacia Fine Chemicals Div. of Pharmacia Inc., Piscataway, N. J.), they were then dialyzed against phosphate-buffered saline (PBS), pH 7.2, aliquoted, and stored at -70°C until use. Before use, these IgG preparations were absorbed with spleen cells of selected strains shown in Tables II and IV.

F(ab')2 fragments of goat anti-rabbit Fab, F(ab')2 of rabbit anti-mouse Fab, and F(ab')2 of rabbit anti-mouse Thy-1 antigen (13) were kindly supplied by Dr. M. Letarte, Department of Medical Biophysics, University of Toronto. These preparations were conjugated to either fluorescein isothiocyanate (FITC) or tetramethyl rhodamine isothiocyanate (TRITC) (British Drug Houses Ltd., Poole, England) during dialysis against these fluorochromes. Briefly, samples at a concentration of 15-20 mg/ml were dialyzed overnight at 4°C against FITC or TRITC at a concentration of 0.1 mg/ml in 0.05 M NaHCO3, pH 9.5. To remove unbound FITC and TRITC, samples were further dialyzed against 0.01 M TRIS-HCl, pH 7.6, containing 0.01 M NaCl and 0.01% Na azide.

Anti-Ly 1.2 and anti-Ly 2.2 sera were characterized and kindly supplied by Dr. I. F. C. McKenzie, Department of Medicine, Austin Hospital, Heidelberg, Victoria, Australia.

Complement. The source of complement was normal rabbit serum that was absorbed with EDTA-agarose and then spleen cells and thymocytes.

**Production of Alloactivated T Cells by GVHR.** Alloactivated T cells were obtained as previously described (8). Briefly, 10⁶ donor thymocytes from mice (4-8 wk old) were injected i.v. into irradiated (800 rads) recipients (8-12 wk old) of matched sex. Recipient spleen cells were recovered 5 d later (viability as estimated by trypan blue exclusion was 70-80%), and dead cells were removed by centrifugation on a 100% heat-inactivated fetal calf serum (FCS) gradient. Using immunofluorescence, >95% of live cells recovered were serologically found to be T cells which expressed H-2(H-2K and H-2D) antigens of the donor haplotype.

**Treatment of Cells with Anti-Ly and C'.** Optimal conditions of treatment were determined from pilot experiments. Cells were incubated with anti-Ly 1.2 (dilution 1:10) or anti-Ly 2.2 (dilution 1:25) for 30 min at room temperature. They were then centrifuged and resuspended in C' (dilution 1:25) for 45 rain at 37°C. The percent lysis achieved with these antisera was assessed by trypan blue exclusion. Treatment with anti-Ly 1.2 lysed 35-40% of alloactivated cells whereas anti-Ly 2.2 lysed 25-30% of cells above the normal mouse serum (NMS) and C' control.

**Immunofluorescence.** Samples containing 2.5 x 10⁶ cells each in PBS-5% FCS were treated for 15 min at 4°C with 50 µl of the IgG fractions of either anti-H-2 (2.1 mg/ml) or anti-Ia (1.8 mg/ml) diluted 1:3 with PBS containing 5% FCS and 0.01% Na azide, as previously reported (14). Cells were then centrifuged through 100% heat-inactivated FCS, washed once, and treated with 50 µl of either FITC or TRITC-conjugated F(ab')2 of rabbit anti-mouse Fab (0.4 mg/ml) diluted 1:50 with PBS-5% FCS-0.01% Na azide. They were incubated and washed as above and prepared for analysis. Staining for Thy-1.2 positive cells was similarly performed using rabbit anti-Thy-1 (14) and TRITC-conjugated F(ab')2 of goat anti-rabbit Fab (0.25 mg/ml).

Approximately 200 cells in each preparation were scored for fluorescence using a Leitz Orthoplan fluorescence microscope, (Wild Leitz Canada Ltd., Willowdale, Ontario) equipped with a Ploemopak 2 illuminator and HBO 50 watt ultra high pressure mercury lamp.

**Suppression of GVHR with Anti-Ia Serum.** Irradiated (800 rads) recipient B10.AQR mice (age 8-11 wk) were injected i.v. with 5 x 10⁷ thymocytes and 5 x 10⁸ spleen cells from donor B10.T(6R) mice (age 6-8 wk) of the same sex. Recipients were divided into four groups (A-D). Group A mice were injected intraperitoneally (i.p.) daily for 7 d with 100 µl (diluted 1:2) of normal mouse serum (NMS). The first serum injection was 8 h before transfer of allogeneic cells. Group B and C mice received 10 and 100 µl of A.TH anti-A.TL (anti-Ia, S₄, G₄), respectively, according to the same schedule as group A. A fourth group (D) received only irradiation and neither allogeneic cells nor antiserum. Mice were injected with serum and weighed daily. At day 7 after the transfer of donor cells, all recipient mice were sacrificed and the ratio of spleen weight to body weight was determined for each mouse and expressed according to the following spleen weight index (15):
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Table II

GVHR Across a Whole H-2 Complex Incompatibility *

| Donor          | Recipient | H-2 Incompatibility | H-2 Regions detected by antisera$ | Percent donor lymphocytes stained |
|----------------|-----------|----------------------|-----------------------------------|---------------------------------|
| B10            | B10.BR    | H-2$^b$              | K$^a$, D$^b$                      | 40                              |
| B10            | B10.BR    | H-2$^b$              | F$^a$, S$^a$, G$^a$               | 50                              |
| B10            | B10.BR    | H-2$^b$              | F$^a$, S$^a$, G$^a$               | 4                               |
| B10.S(7R)      | B10.BR    | H-2$^b$              | K$^a$, D$^b$                      | 33                              |
| B10.S(7R)      | B10.BR    | H-2$^b$              | F$^a$, S$^a$, G$^a$               | 38                              |
| B10.S(7R)      | B10.BR    | H-2$^b$              | F$^a$, S$^a$, G$^a$               | 4                               |
| B10.S(7R)      | B10.BR    | H-2$^b$              | I$^a$, B$^a$, I-J$^b$             | 36                              |
| B10.S(7R)      | B10.BR    | H-2$^b$              | E$^a$, C$^a$, S$^b$, G$^a$        | 2                               |
| B10.S(7R)      | B10.BR    | H-2$^b$              | J$^b$                            | 4                               |
| B10.BR         | (B10.BR × B10.S)F$^b$ | H-2$^b$              | F$^a$, S$^a$, G$^a$               | 5                               |
| B10.BR         | (B10.BR × B10.S)F$^b$ | H-2$^b$              | F$^a$, S$^a$, G$^a$               | 30                              |
| B10.BR × B10.S | B10.BR    | None                 | F$^a$, S$^a$, G$^a$               | 8                               |
| B10.BR × B10.S | B10.BR    | None                 | F$^a$, S$^a$, G$^a$               | 5                               |

* See text for explanation.
$\dagger$ Anti-K$^a$, D$^b$ was produced by extensive absorption of C3H.SW anti-C3H/DiSn (anti-H-2$^b$) with A.TL spleen cells as determined by the dye exclusion microcystocytotoxic assay (16). Before use, anti-K$^a$, D$^b$, anti-I-A$^a$, I-B$^a$, I-J$^b$, anti-I$^a$, S$^a$, G$^a$, anti-I-E$^a$, I-C$^a$, S$^b$, G$^a$, and anti-I-F$^a$ were absorbed with donor B10, B10.S(7R), and B10.T(6R) spleen cells. Anti-I$^a$, S$^a$, G$^a$ was absorbed with A.TL, B10, and B10.T(6R) spleen cells. Absorptions were performed by incubating 150 μl of serum twice with 5 × 10$^7$ cells of a given strain for 30 min at 4°C. Sera were spun at 100,000 g for 1 h immediately before use to remove possible aggregates.
§ Donor cells were injected into B10.T(6R) recipients.

A statistical analysis was carried out using the Student’s t test.

Results

GVHR across Entire H-2-Region Differences. Various strain combinations were used to activate donor cells during a GVHR against H-2-associated alloantigens of the recipient. It was of interest to determine whether alloactivated donor T cells can acquire, in vivo, H-2 and Ia antigens from the surface of recipient cells. This was examined by the immunofluorescent staining of these donor cells with alloantisera (rendered specific by absorption) directed against the recipient H-2 haplotype. Representative results are shown in Table II. Control values were obtained by the injection of donor cells into B10.T(6R) hosts, which carry an H-2 haplotype different from that of the specific recipient haplotype. With the B10(H-2$^b$): B10.BR(H-2$^b$) donor; recipient combination, 40% of the cells recovered from the recipient spleen (>95% donor haplotype when stained with C3H/DiSn anti-C3H.SW [anti-H-2$^b$]) stained with the anti-K$^a$, D$^b$ antisera. An example of such a stained cell is shown in Fig. 1. Approximately 50% of the recovered cells were stained with anti-I$^a$, S$^b$, G$^a$. By contrast, control samples yielded <10% staining. Another combination which employed B10.S(7R) donor cells and B10.BR recipient cells demonstrated that ≈33% of donor cells were stained with anti-K$^b$, D$^b$, whereas 38% and 36% of these cells were stained with anti-I$^a$, S$^b$, G$^a$, and anti-I-A$^b$, I-B$^b$, I-J$^b$, respectively. No positive staining
Fig. 1. Immunofluorescence of a donor blast T cell. The photo on the top (A) shows donor blast T cells stained with F(ab')₂ fragments of rabbit anti-mouse Thy-1 and TRITC-conjugated F(ab')₂ fragments of goat anti-rabbit Fab. The photo on the bottom (B) shows a donor blast T cell stained with anti-recipent anti-la serum and FITC-conjugated F(ab')₂ fragments of rabbit anti-mouse Fab. Magnification, 400 ×.
of these donor cells above the control values (<10%) was observed upon treatment with either anti-I-Jk or anti-I-Ek, I-Cx, Sx, Gx. With the B10.BR-donor and (B10.BR × B10.S)F1-recipient combination, 30% of donor cells were stained with anti-I-Jk, Sx, Gx, although only 5% of these cells were stained with anti-I-Ek, I-Cx, Sx, Gx.

In each of the above combinations, the stimulation of a GVHR across an incompatibility in the whole H-2 complex generated donor cells recovered from recipient spleens that were comprised of 50–60% large T cells or blasts (a blast was considered to have two × diameter of a small lymphocyte (11)). Approximately 70–80% of donor cells that were stained with alloantisera against the recipient H-2 haplotype were identified as T-cell blasts. However, no attempt was made here to distinguish between large and mid-size blasts and small lymphocytes since we found this introduced inconsistency in counting. For this reason, all stained cells were counted in each preparation. In addition, >95% of these lymphocytes were found to express donor haplotype controlled H-2 but not Ia antigens, i.e., these cells were predominantly of donor origin.

The results presented here suggest that recipient-derived H-2K and H-2D antigens and Ia antigens controlled only by the I-A subregion (no known Ia antigenic specificities are controlled by I-B), may be absorbed onto the surface of donor-alloactivated T cells. The lack of staining observed with anti-I-Jk may be due to its relatively low cytotoxic activity (<10% lysis of B10.BR target cells). The same explanation cannot be attributed to the absence of staining seen with anti-I-Ek, I-Cx, Sx, Gx, which lyse 50–60% of B10.BR lymph node cells with a titer of 1:640 in a microcytotoxicity assay (16).

**Effect of Treating GVHR Donor Cells with Anti-Lyt Sera and C'**. Nagy et al. (11) have found that MLR responder T cells of the Lyt-1+, 2−, 3− phenotype adsorb Ia antigens of the stimulator haplotype, whereas responder T cells of the Lyt-1−, 2+, 3+ phenotype adsorb H-2K and H-2D antigens of the stimulator haplotype. The following experiment was performed to determine whether similar subpopulations of GVHR donor T cells acquire host H-2 and Ia antigens. Treatment with anti-Lyt-1.2 and C' of B10 donor cells activated in B10.BR recipients resulted in an increase in the percentage of cells stained with anti-Kx, Dx from 40 to 68% and a decrease in cells stained with anti-I-x, Sx, Gx from 50 to 15% (see Table III). On the other hand, treatment with anti-Lyt-2.2 and C' increased the percent cells stained with anti-I-x, Sx, Gx from 50 to 69% but decreased the percent of cells stained with anti-Kx, Dx from 40 to 11%. These results

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**Table III**

*Anti-Ly Plus C' Treatment of Donor Cells*

| Donor | Recipient | H-2 Incompatibility | Anti-Ly + C' treatment | H-2 regions detected by anti-sera | Percent lymphocytes stained |
|-------|-----------|----------------------|-------------------------|----------------------------------|---------------------------|
| B10   | B10.BR    | H-2k                 | Anti-Ly 1.2             | Kx, Dx                           | 68                        |
| B10   | B10.BR    | H-2k                 | Anti-Ly 1.2             | Pk, Sk, Gx                       | 15                        |
| B10   | B10.BR    | H-2k                 | Anti-Ly 2.2             | Kx, Dx                           | 11                        |
| B10   | B10.BR    | H-2k                 | Anti-Ly 2.2             | Pk, Sk, Gx                       | 69                        |
| B10   | B10.BR    | H-2k                 | —                       | Kx, Dx                           | 40                        |
| B10   | B10.BR    | H-2k                 | —                       | Pk, Sk, Gx                       | 50                        |

* See text for explanation.
† Sera were absorbed as described in the legend to Table II.
GVHR Across an I-Region or I-Subregion Incompatibility

| Donor | Recipient | H-2 Incompatibility | H-2 regions detected by antisera | Percent donor lymphocytes stained | Specific | Control |
|-------|-----------|---------------------|---------------------------------|---------------------------------|----------|---------|
| A.TH  | A.TL      | I\(^{a}\), S\(^{a}\), G\(^{a}\) | I\(^{a}\), S\(^{a}\), G\(^{a}\) | 37% | 3% | 2% | 30% |
| A.TH  | A.TL      | I\(^{a}\), S\(^{a}\), G\(^{a}\) | I\(^{a}\), I-B\(^{b}\), I-J\(^{a}\) | 36% | 7% | 2% | 6% |
| A.TH  | A.TL      | I\(^{a}\), S\(^{a}\), G\(^{a}\) | I-E\(^{a}\), I-C\(^{a}\), S\(^{a}\), G\(^{a}\) | 2% | 6% | 32% | 6% |
| (A.TH × B10.HTT)\(F_{1}\) | A.TL | I-A\(^{b}\), I-B\(^{b}\), I-J\(^{b}\) | I\(^{a}\), S\(^{a}\), G\(^{a}\) | 34% | 6% | 9% | 33% |
| (A.TH × B10.HTT)\(F_{1}\) | A.TL | I-A\(^{b}\), I-B\(^{b}\), I-J\(^{b}\) | I\(^{a}\), S\(^{a}\), G\(^{a}\) | 3% | 6% | 5% | 5% |
| (B10.S(7R)) | B10.HTT | I-E\(^{a}\), I-C\(^{a}\), S\(^{a}\), G\(^{a}\) | I\(^{a}\), S\(^{a}\), G\(^{a}\) | 16% | 3% | 7% | 7% |
| (B10.S(7R)) | B10.HTT | I-E\(^{a}\), I-C\(^{a}\), S\(^{a}\), G\(^{a}\) | I\(^{a}\), I-C\(^{a}\), S\(^{a}\), G\(^{a}\) | 7% | 6% | 3% | 7% |
| (B10.A(3R)) | B10(A[5R]) | I-J\(^{b}\) | I\(^{a}\), S\(^{a}\), G\(^{a}\) | 8% | 8% | 6% | 6% |

* See text for explanation.

† Absorption of anti-I\(^{a}\), S\(^{a}\), G\(^{a}\); anti-I-A\(^{b}\), I-B\(^{b}\), I-J\(^{b}\); anti-I-E\(^{a}\), I-C\(^{a}\), S\(^{a}\), G\(^{a}\); and anti-I-J\(^{b}\) were performed with A.TH and B10.S(7R) spleen cells. Anti-I-A\(^{b}\), I-B\(^{b}\), I-J\(^{b}\) was absorbed with A.TL and B10 spleen cells. Absorptions were performed as described in the legend to Table I.

§ Controls consisted of reciprocal combinations, i.e., recipient and donor strains were interchanged.

parallel those previously obtained with MLR-responder T cells mentioned above and are consistent with a similar Ly phenotype and function for GVHR-activated donor T cells.

**GVHR across I-Region and I-Subregion Differences.** Donor cells were treated with anti-I\(^{a}\) antisera directed against different I-subregions of the recipient haplotype in an attempt to map the I\(^{a}\) loci coding for the I\(^{a}\) antigens which become adsorbed onto the donor T cells. Typical results are presented in Table IV. Control values were obtained with the reciprocal combinations of those used in the test combinations, i.e., recipient and donor strains were interchanged. In the A.TH-A.TL combination, which leads to an activation across the I\(^{a}\) region, 37% of A.TH donor cells were stained with anti-I\(^{a}\), S\(^{a}\), G\(^{a}\) and 36% of the cells were stained with anti-I-A\(^{b}\), I-B\(^{b}\), I-J\(^{b}\). No positive staining was observed with either anti-I-E\(^{a}\), I-C\(^{a}\), S\(^{a}\), G\(^{a}\), or anti-I-J\(^{b}\) or anti-I-J\(^{b}\). In the reciprocal combination, 30% of A.TL donor cells were stained with anti-I\(^{a}\), S\(^{a}\), G\(^{a}\), although anti-I-J\(^{b}\), S\(^{a}\), G\(^{a}\) yielded background level staining. Similar results to those described above were obtained by activation across the I-A\(^{b}\), I-B\(^{b}\), and I-J\(^{b}\) subregions in the (A.TH × B10.HTT)\(F_{1}\)-donor and A.TL-recipient combination.

By contrast, activation across either I-E\(^{a}\), I-C\(^{a}\), S\(^{a}\), and G\(^{a}\) (B10.S[7R]—donor and B10.HTT—recipient) or across I-J\(^{b}\) (B10.A[3R]—donor and B10.A[5R]—recipient) yielded a background level of staining with all antisera used.

All combinations which involved an I-A subregion incompatibility gave rise to donor cells composed of 40–50% blast cells. These blasts represented 70–80% of the positively stained cells. However, activation across either I-J or I-E, I-C, S, and G differences yielded <5% blasts. Positive results therefore correlated closely with the presence of a large number of blasts resulting from a strong GVHR.
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Fig. 2. Suppression of the GVHR with anti-host anti-Ia serum. Groups A, B, and C represent B10.AQR irradiated mice that received an i.v. injection of B10.T(6R) spleen cells and thymocytes. Group D mice received only irradiation. Beginning on the day of allogeneic cell transfer, group A mice received 100 µl of NMS i.p. daily and groups B and C received 10 µl and 100 µl of anti-Ia* serum, respectively. At day 7, all mice were sacrificed and the spleen to body weight ratio was determined for each mouse. Numbers in parentheses represent the number of mice used per group.

Suppression of GVHR by Injection of Anti-Ia Serum to Host Antigens. Previous reports demonstrated both in clinical studies (17) and animal model systems (18-22) that anti-recipient antibodies can suppress the GVHR. However, the reactivity of these sera versus specific MHC products was not shown. The following experiment was therefore performed to determine if an anti-Ia antiserum to recipient Ia antigens could suppress the GVHR. The combination used here consisted of B10.T(6R) donors and B10.AQR recipients, which results in the activation across the I-A\(^k\), I-B\(^k\), I-J\(^k\), I-E\(^k\), I-C\(^k\), S\(^a\), and G\(^a\) regions. Recipient mice were administered daily i.p. injections of either anti-I\(^k\), S\(^k\), G\(^k\), or A.TH NMS. Their spleen weight indices were evaluated 7 d after irradiation and cell transfer. As can be seen from Fig. 2, mice undergoing GVHR that received 100 µl of NMS (group A) had a marked splenic enlargement (spleen weight index = 7.64). Group B mice, which received 10 µl of anti-I\(^k\), S\(^k\), G\(^k\), had significantly smaller spleens (spleen weight index = 5.56; B < A, P < 0.001). Group C mice which received 100 µl of anti-I\(^k\), S\(^k\), G\(^k\) had the smallest spleens of the three groups (spleen weight index = 4.14; C < B < A, P < 0.001) as they possessed average spleen weights almost one-half that of group A. The control Group D mice which received only irradiation and no allogeneic cells had still smaller spleens (spleen weight index = 1.15).

These data indicate that the administration of recipients with alloantisera directed against their Ia antigens can markedly suppress an ongoing GVHR response. Whether this suppression occurs via the interaction of anti-Ia with either the irradiated recipient cells or the transferred donor cells which have absorbed recipient Ia antigens, or both of these cell populations, remains to be determined.

Discussion

Data presented here establish a definite parallelism between the functional activity of alloactivated T cells responsive in vitro during an MLR and those responsive in vivo during a GVHR. Nagy et al. (10, 11) previously reported that different subpopulations of MLR responder T-cell blasts acquire either K- and D-region products or I-region products from the irradiated stimulator cells. However, these investigators did not identify whether all or only certain stimulator cell derived I-region products
appear on the surface of the responder T cells. In this study, we have shown that GVHR donor cells can absorb K-, D- and I-region products from irradiated recipient cells. Furthermore, we have demonstrated that only host cell products of the I-A and/or I-B subregion (presumably only the I-A subregion is involved here) are identifiable on donor cells. These results are consistent with the finding that an I-A subregion difference leads to a strong stimulation of the GVHR (3, 5, 6), whereas an I-C subregion difference causes only a relatively weak stimulation that requires a presensitization of the donor cells (5, 6). I-B and I-J differences do not stimulate a GVHR response (5). Whether recipient H-2 and Ia antigens bind to activated donor cells either directly to their surface or indirectly via the interaction with specific cell surface receptors remains to be determined. In each instance, this type of binding must occur in such a manner that the alldeterminants of these molecules must be exposed for reactivity with their respective antibodies.

The uptake by donor cells of host MHC gene products may also have an important physiological role in immune surveillance and protection. Such an hypothesis has been suggested to explain the protection of lung Schistosoma mansoni schistosomula from immune destruction resulting from the binding to these organisms of the infected allogeneic recipient H-2 antigens (23). It is possible that such a mechanism may also immunologically protect fetal cells in the maternal circulation by the acquisition of paternal MHC gene products by the maternal cells.

Cantor and Boyse (24, 25) have determined the Ly phenotype of two different lymphocyte subpopulations functional in an MLR. They have reported that Ly-1+, 2-, 3- responder T cells recognize Ia antigens, whereas Ly-1-, 2+, 3+ responder T cells recognize H-2K and H-2D antigens. Nagy et al. (11) found that MLR-stimulator cell Ia antigens bind to Ly-1+, 2-, 3- responder T cells, whereas H-2K antigens of the stimulator cell bind to Ly-1-, 2+, 3+ responder T cells. Our studies on GVHR activated donor cells have corroborated these MLR data. We have shown that treatment of GVHR alloactivated donor T cells with anti-Ly-1.2 and C' significantly reduced the percentage of the surviving viable cells stained with antisera against recipient Ia antigens but increased considerably the percentage of cells stained with an antiseraum against recipient H-2K and H-2D antigens. Treatment of the activated donor cells with anti-Ly-2.2 and C' yielded the opposite result. It should also be noted that Eshhar et al. (26) found that pretreatment with anti-Ly-1.2 plus C' of the MLR responder cells used to produce an AEF resulted in their inability to obtain any T-cell replacing activity with such an AEF. It therefore appears that the Ly phenotype of donor T cells responsive during a GVHR is the same as that of the responder T cells in an MLR.

The results obtained in this study serve to further delineate the role of Ia antigens in the GVHR response. The daily injection of irradiated recipients with an antiserum against self Ia antigens produced a marked suppression of the resulting GVHR. The degree of suppression observed was dependent on the dose of anti-Ia administered. It should be mentioned that no attempt was made here to determine the optimum kinetics and dose of administration of anti-Ia antisera. In addition, it is not yet known whether this type of suppression can occur via the binding of such an anti-Ia antibody either to the surface of the transferred donor cells which have absorbed recipient Ia antigens or directly to the surface of the irradiated recipient cells, or perhaps to both of these cell populations. Anti-Ia antisera reactive with I-J determinants may eliminate suppressor T-cell function in vivo (27), whereas antisera against I-A determinants may
eliminate helper T-cell function in vivo (28). Because recipient Ia antigens determined by the I-A subregion appear to be absorbed to GVHR activated donor, Ly-1+, 2−, 3− T cells, it is possible that the anti-Ia antiserum used here, which contains anti-I-A activity, suppressed the GVHR in part by binding to and abrogating the function of this subpopulation of donor T cells. Conversely, it is also likely that a GVHR response may be suppressed by the reaction of anti-recipient Ia antibodies with recipient cells. This suggestion is based on the observations that (a) MLR (29-33) and CML (34) responses may be inhibited by anti-Ia antisera directed against the MLR stimulator and CML target cells, respectively, and (b) skin (35), heart, and kidney (36) allograft survival may be enhanced by treatment of transplant recipients with anti-donor anti-Ia antisera.

Anti-recipient alloantibodies have previously been used to suppress a GVHR response (6, 18-22), but only one such report documented the use of an anti-Ia serum (6). In this instance, however, a single injection of anti-recipient Ia antibodies was given on day 0 to donors and not to recipients. It was found that if these donor cells were recovered within 8-14 d after serum pretreatment, they produced a weaker GVHR when subsequently injected into newborn mice. Only the donor spleen cells, but not the donor lymph node cells, were suppressed. The use of antisera to either I-A or I-C determinants resulted in an equivalent amount of suppression. The mechanism for this type of suppression is not clear in view of the relatively low doses of antisera injected, the similar degree of suppression obtained with anti-I-A and anti-I-C, and the short duration of the induced suppression.

The ability (a) to detect recipient Ia antigens on activated donor cells during a GVHR response and (b) to suppress such an alloactivation with anti-recipient Ia antibodies may provide further insight into the mechanism(s) of allogeneic lymphocyte interactions. A central role for Ia antigens in the regulation of lymphocyte interaction during an immune response has been suggested (reviewed in references 38-40). Based on our studies with AEF, we have proposed that Ia antigens represent a second signal (immunizing antigen represents the first signal) required for the activation of a B cell to antibody production (8, 9). The capacity of GVHR and MLR alloactivated T cells to acquire stimulator cell Ia antigens on their surface may explain why the helper activity of an Ia-positive AEF generated across various I-subregion differences is restricted to B cells of the stimulator haplotype (9). AEF preparations which consist of Ia antigens determined only by the stimulator haplotype provide little or no help for B cells of the responder haplotype (8). This H-2 restriction of AEF activity may therefore be controlled by the recognition and like-like interaction between AEF Ia antigens, which are initially stimulator cell derived and are then adsorbed onto allogeneic responder T cells, and syngeneic Ia antigens on the B cell. Alternatively, AEF Ia antigens may recognize and bind to complementary anti-Ia receptors on the B cell. It is also possible that after antigenic stimulation, T cells may similarly acquire B-cell surface derived Ia antigens. In this way, T cells may recruit and cooperate with the appropriate syngeneic macrophages and B cells to activate these B cells to antibody synthesis.

Ia antigens may also serve as a second signal in an MLR and GVHR response. For example, Ia antigens may act as both the stimulating antigen and the second signal

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required for either MLR responder T cell or GVHR donor T-cell activation. In the absence of transfer of Ia antigens from stimulator to responder cells alloactivation may not occur. This view is consistent with the finding that K- and D-region differences alone stimulate a weak MLR (42) and a weak GVHR (3). It is also compatible with the finding that the removal of K- and D-region reactivity by absorption with erythrocytes from allograft enhancing anti-H-2 sera does not reduce their enhancing activity (36, 37). In addition, the recent data of Batchelor et al. (42) show that H-2 and Ia antigens are poor immunogens unless presented on viable cells. Presumably, metabolically active cells are needed to relay a secondary signal. Anti-Ia antibodies to stimulator alloantigens may then suppress alloactivation by interfering with the transfer of Ia antigens from stimulator to responder lymphocytes.

Although precise mechanisms of action remain uncertain, it is becoming increasingly evident that the in vivo injection of anti-Ia antibodies may provide a powerful research and clinical tool for immunomanipulation. In the clinical situation, antisera to HLA D-region antigens (the human analogue of murine Ia antigens) may prove valuable for the prevention or treatment of GVHD in bone marrow transplant recipients.

Summary

By using an indirect immunofluorescence technique, the presence of host cell derived H-2K, H-2D, and Ia alloantigens on donor cells recovered from recipient spleens after a graft-versus-host response (GVHR) was demonstrated. Mapping studies indicated that only host K, D, and I-A region gene products could be identified on the donor cells. Host I-E/C- and I-J-subregion products were not absorbed by donor cells.

Treatment of activated donor cells with anti-Ly sera plus C' revealed that donor cells carrying host Ia antigens have a Ly-1+, 2−, 3− phenotype, whereas donor cells carrying H-2K and H-2D host antigens have a Ly-1−, 2+, 3+ phenotype.

A GVHR that resulted from only an I-region incompatibility was suppressed by the injection of recipient mice with an anti-Ia antiserum directed against self Ia antigens. The degree of suppression was proportional to the amount of anti-Ia antiserum administered.

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