ALLOSUPPRESSOR AND ALLOHELPER T CELLS IN ACUTE AND CHRONIC GRAFT-VS.-HOST DISEASE

II. F1 Recipients Carrying Mutations at H-2K and/or I-A*

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A systemic graft-vs.-host reaction (GVHR) induced in nonirradiated recipients can give rise to a variety of pathological symptoms. One of the possible outcomes is acute GVH disease (GVHD). After an initial brief phase of lymphoid stimulation (1-3), acute GVHD rapidly produces suppressive pathological symptoms, such as pancytopenia accompanied by aplastic anemia and hypogammaglobulinemia (1-5). A different possible consequence of the GVHR is stimulatory GVHD, also referred to as chronic GVHD. The symptoms of chronic GVHD include a persistent lymphoid hyperplasia (1-4, 6), hypergammaglobulinemia (2-4, 7, 8), and the formation of autoantibodies and pathological lesions reminiscent of systemic lupus erythematosus (SLE) and other types of vascular collagen disease (2, 3, 6, 8, 9).

Both types of GVHD can be experimentally induced in nonirradiated F1 hybrid recipients by the injection of lymphocytes from one of the parental strains. A common requirement for the induction of both acute and chronic GVHD is the presence of T lymphocytes in the donor-cell inoculum (3-5, 8, 9). Which type of GVHD will develop after the injection of parental T cells appears to depend on the functional subset of donor T cells activated in the F1 host. Work from this laboratory has shown that the stimulatory type of GVHD can be induced by alloreactive donor T helper (Th) cells (2-5, 8, 9) carrying the Lyt-1+2- phenotype, whereas the induction of acute GVHD requires both alloreactive Th and T suppressor (Ts) cells (2-5). In the development of acute GVHD, there appears to be a sequential activation of these two subsets of donor T cells: first, alloreactive Th cells induce the brief initial phase of...
stimulatory symptoms (week 1) and, then (week 2) help to activate the alloreactive T\textsubscript{S} cells (2, 3). The alloreactive donor T\textsubscript{S} cells appear to be the final effector cells causing the rapidly developing suppressive symptoms of acute GVHD (2-5).

Apart from the requirement of donor T cells, another common denominator for the induction of both types of GVHD is that an H-2 incompatibility between donor and nonirradiated F\textsubscript{1} host is required (3, 5).\textsuperscript{2} Several groups have studied the intra-H-2 differences required for the induction of GVHR (10-15). These studies were limited, however, to the use of the popliteal lymph node weight assay (15) and the spleen weight assay (11-14), which were performed at a single point in time, and to the mortality assay (12-15). The results obtained show that a strong spleen weight increase as well as a strong popliteal lymph node weight increase is induced across an I region difference and that only a weak or moderate increase is induced across K or D region incompatibilities (10, 14). Using irradiated F\textsubscript{1} mice as recipients, the mortality rate was found to be maximal across a total H-2 difference (15). Moderate mortality rates were found when donor and host differed at either K, I, or D alone (15).

From studies on the primary antibody response in vitro, on the other hand, it is known that class II (I-A/I-E) alloantigens preferentially trigger alloreactive T\textsubscript{S} cells, which cause the positive allogeneic effect (16),\textsuperscript{4} and that class-I (K/D) alloantigens preferentially trigger alloreactive T\textsubscript{S} cells, causing the negative allogeneic effect (16).\textsuperscript{4}

In the present investigation, using GVH assays that are able to measure both allohelp and allosuppression, we studied whether the functional dichotomy between class I and class II alloantigens exists also in vivo. We asked whether well-defined differences in class I and/or class II alloantigens can selectively activate, out of a pool of unselected donor cells, the subpopulation(s) of T cells that cause either stimulatory or suppressive GVHD. The strains of mice used for this study were C57BL/6 (B6), the B6 mutant strain B6.C-H-2\textsuperscript{bml} (bm1), which carries a mutation at the K locus (17), and the B6 mutant strain B6.C-H-2\textsuperscript{bml2} (bm12), which carries a mutation at the I-A locus of H-2 (18). The results show that, indeed, the dichotomy observed in vitro (16)\textsuperscript{4} also exists in vivo. An exclusively stimulatory GVHD was induced across a difference at I-A alone, whereas the induction of suppressive GVHD required a difference at both I-A and K. By contrast, the K locus difference alone was insufficient for triggering clear-cut symptoms of either stimulatory or suppressive GVHD.

Materials and Methods

Mice. B6 mice were purchased from Olac 1976 Ltd. (Bicester, Oxon, England). The H-2K\textsuperscript{b} mutant strain bm1 (17) was originally obtained from Dr. D. W. Bailey (The Jackson Laboratory, Bar Harbor, ME). The H-2 I-A\textsuperscript{b} mutant strain bm12 (18) was originally obtained from Dr. R. W. Melvold (Northwestern University, Chicago, IL). Both strains were maintained at our laboratory by sibling-to-sibling matings. The F\textsubscript{1} hybrid mice (B6 × bm1), (B6 × bm12), and (bm1 × bm12) were bred in our animal facilities. Sex-matched donor and recipient mice, 8-12 wk old, were used.

Preparation of Donor Cells. Single-cell suspensions of spleen and lymph node cells of B6 donors were prepared as described (5).

Induction of GVHR. In experiment I, (B6 × bm1)F\textsubscript{1}, (B6 × bm12)F\textsubscript{1}, and (bm1 × bm12)F\textsubscript{1} hybrid mice received a total of 10\textsuperscript{8} viable B6 cells consisting of one-third lymph node cells and

\textsuperscript{4} Rolink, A. G., W. van der Meer, C. J. M. Melief, and E. Gleichmann. 1983. Intra-H-2 and T cell requirements for the induction of maximal positive and negative allogeneic effects in vitro. Eur. J. Immunol. In press.
two-thirds spleen cells; these cells were administered intravenously in two equal dosages on
and 7. In experiment II, the three kinds of F1 hybrid mice received a single intravenous
jection of $10^9$ viable B6 spleen cells. Age- and sex-matched noninjected F1 (N F1) mice of the
three strains were used as controls.

**Protein A Plaque-forming Cell Assay.** To determine the total number of Ig-secreting cells in the
spleens of N F1 mice and in the spleens of F1 mice undergoing a GVHR (GVH F1), a
modification of the protein-A plaque assay was performed as described (19).

**Primary In Vitro Anti-sheep Erythrocyte (SRBC) Response.** On day 0, a constant number of $5 \times 10^8$ spleen cells obtained from N F1 animals were co-cultured with SRBC and increasing
numbers of spleen cells derived from GVH F1 mice (2, 20). Cultures were performed in Falcon
multi-well tissue-culture plates (3008; Falcon Labware, Oxnard, CA) containing 500 µl of
culture medium. The latter consisted of Iscove's modified Dulbecco's medium supplemented
with 10% fetal calf serum, penicillin (50 IU/ml), streptomycin (50 IU/ml), and 2-mercaptoeth-
anol ($2 \times 10^{-5}$ M).

**Clinical Signs of GVHR.** Twice a week, the GVH F1 were inspected for symptoms of acute
GVHD, such as weight loss, diarrhea, and ruffled fur. The number of dead mice was recorded.
Anemia was determined by biweekly measurement of hematocrit. At weekly intervals, the
GVH F1 mice were tested for elevated proteinuria ($\geq 300$ mg protein per 100 ml urine) by
means of Albustix test sticks (2872; Miles Laboratories, Ames Div., Elkhart, IN). Previously it
has been shown (21) that elevated proteinuria is a reliable indicator of immune-complex
glomerulonephritis (ICGN) in GVH F1 mice.

**Detection of Autoantibodies.** IgG autoantibodies to erythrocytes were detected by a direct
Coombs' test using a 1:100 diluted, heat-inactivated rabbit-anti-mouse IgG serum, as described
(8). Serum autoantibodies to thymocytes were detected by a complement-dependent cytotoxicity
test (8), in which $^{35}$Cr-labeled thymocytes from normal B6 mice were used as target cells. Mice
were scored positive for autoantibodies against thymocytes when a 1:5 dilution of their serum
lysed $\geq 40\%$ of the target cells. Serum IgG antibodies against nuclear antigens were detected by
an indirect immunofluorescence technique, applying fluorescein-labeled goat-anti-mouse IgG
serum; cryostat sections of mouse liver were used as antigenic substrate, as described (8). Serum
IgG autoantibodies against double-stranded DNA (dsDNA) were determined by an indirect
immunofluorescence technique applying a fluorescein-labeled goat-anti-mouse IgG (Meloy
Laboratories Inc., Springfield, VA); the extranuclear dsDNA of the hemoflagellate
*Crithidia luciliae* was used as antigenic substrate, as described (8, 22). For the determination of both
anti-nuclear and anti-dsDNA antibodies, the initial serum dilution was 1:10; the highest serum
dilution at which specific immunofluorescence was seen was called the titer.

**Determination of ICGN.** At autopsy, one kidney of each GVH F1 hybrid was snap-frozen and
stored in liquid nitrogen. Cryosections, 4 µm thick, were prepared and incubated for 30 min
with a 1:60 dilution of rabbit-anti-mouse IgG labeled with fluorescein isothiocyanate (KM 16-
11-F3; Central Laboratory of the Netherlands Red Cross Blood Transfusion Service). After a
wash with phosphate-buffered saline for 30 min, the sections were mounted with glycerol,
diluted 1:2 phosphate-buffered saline, and examined in a Leitz Orthoplan microscope.

**Statistical Significance.** Student's $t$ test was applied.

**Results**

**The Capacity of B6 Donor Cells to Induce Lethal GVHD (LGVHD) Requires a Difference
in the F1 Recipients at Both I-A and K.** Groups of nonirradiated (bm1 × bm12)F1, (B6
× bm1)F1, and (B6 × bm12)F1 mice were injected intravenously with $10^9$ B6 cells; the
donor-cell inoculum consisted of two parts of spleen and one part of lymph node cells.
After initiation of the GVHR, the (bm1 × bm12)F1 recipients, which differ from the
B6 donor strain by mutations at both the K and I-A locus, all developed the
characteristic symptoms of acute GVHD, including a reduced body weight and
reduced hematocrit values (Table I), and died (Fig. 1). In marked contrast, none of the
(B6 × bm1)F1 recipients, which differ from the B6 donor strain by a mutation at
the K locus alone, died from or showed clinical symptoms of acute GVHD (Fig. 1,
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Table I
Body Weight and Hematocrit Values of the Three Kinds of F1 Mice at Different Times after the Induction of GVHR

| GVH F1 mice tested and H-2 difference indicated† | Weeks after the initiation of GVHR |   |   |   |
|------------------------------------------------|----------------------------------|---|---|---|
|                                                 | 19 | 3 | 8 |
| Mean body weight ± SEM | Mean hematocrit ± SEM | Mean body weight ± SEM | Mean hematocrit ± SEM | Mean body weight ± SEM | Mean hematocrit ± SEM |
| (bml × bm12)F1 K + I-A | 20.5 ± 0.4 | 43.4 ± 0.2 | 17.0 ± 0.5§ | 34.4 ± 0.6§ |
| (B6 × bm1)F1 K | 20.0 ± 0.7 | 42.7 ± 0.6 | 22.0 ± 0.6† | 43.0 ± 0.3** |
| (B6 × bm12)F1 I-A | 20.1 ± 0.6 | 42.1 ± 0.2 | 22.7 ± 0.3† | 42.3 ± 0.4** |

* 1 wk before the initiation of GVHR.
† GVHR was induced by intravenous injection on days 0 and 7 of a mixture of 5 × 10⁷ live B6 donor cells containing one-third lymph node cells and two-thirds spleen cells.
§ Significantly lower (P < 0.05) when compared with the corresponding values at week -1.
¶ All (bml × bm12)F1 recipients had died from acute GVHD by this time (Fig. 1).
** Not significantly different (P > 0.05) from the corresponding values at week -1.

Table I). Likewise, none of the (B6 × bm12)F1 recipients, which differ from the B6 donor strain by a mutation at the I-A locus alone, died from or showed clinical symptoms of acute GVHD (Fig. 1, Table I).

The Capacity of B6 Donor Cells to Induce a Systemic Lupus Erythematosus (SLE)-like Stimulatory GVHD Requires a Difference in the F1 Recipients at I-A Alone. The same group of nonirradiated F1 recipients studied for the induction of LGVHD, were tested for the presence of SLE-like autoantibodies in the serum, elevated proteinuria as an indication of severe ICGN (21), and immunohistological evidence of ICGN.

1 wk before the induction of the GVHR, none of the F1 mice had detectable amounts of autoantibodies in their serum or elevated proteinuria (Table II). In the
### Table II

Different Formation of SLE-like Autoantibodies and Different Development of Proteinuria in the Three Kinds of GVH F1 Mice

| Weeks after first injection of B6 donor cells* | Number of F1 mice tested | Erythrocytes | Thymocytes | Nuclear antigens | dsDNA | Percentage of F1 mice with elevated proteinuria |
|-----------------------------------------------|--------------------------|--------------|------------|-----------------|-------|-------------------------------------------|
|                                               |                          | Percent at time indicated | Cumulative percent | Percent at time indicated | Cumulative percent | Mean logarithm of positive mice | Percent at time indicated | Cumulative percent | Mean logarithm of positive mice |
| Recipient (bml × bm12)F1 (K plus I-A difference) |                          |                           |                 |                  |     |                                          |                           |                 |                                          |
| -1‡                                           |                          | 15                         | 0               | 0                | 0                | <1                          | 0                           | 0                | <1                          | 0 |
| 2                                             |                          | 15                         | 0               | 0                | 0                | <1                          | 0                           | 0                | <1                          | 0 |
| 4                                             |                          | 0                          | -               | -                | -                | -                           | -                           | -                | -                           | - |
| Recipient (B6 × bm1)F1 (K difference)         |                          | 13                         | 0               | 0                | 0                | <1                          | 0                           | 0                | <1                          | 0 |
| -1‡                                           |                          | 14                         | 0               | 0                | 0                | <1                          | 0                           | 0                | <1                          | 0 |
| 2                                             |                          | 14                         | 0               | 0                | 0                | <1                          | 0                           | 0                | <1                          | 0 |
| 4                                             |                          | 14                         | 0               | 0                | 0                | <1                          | 0                           | 0                | <1                          | 0 |
| 8                                             |                          | 13                         | 0               | 0                | 92               | 92                          | 83                          | 83               | 1.9                         | 31 |
| 14                                            |                          | 12                         | 0               | 0                | 83               | 83                          | 83                          | 83               | 1.6                         | 31 |
| Recipient (B6 × bm12)F1 (I-A difference)      |                          | 13                         | 0               | 0                | 0                | <1                          | 0                           | 0                | <1                          | 0 |
| -1‡                                           |                          | 12                         | 0               | 0                | 92               | 92                          | 83                          | 83               | 1.9                         | 31 |
| 2                                             |                          | 13                         | 0               | 0                | 100              | 100                         | 83                          | 83               | 1.6                         | 31 |
| 4                                             |                          | 13                         | 0               | 0                | 100              | 100                         | 83                          | 83               | 1.9                         | 31 |
| 8                                             |                          | 12                         | 42              | 42               | 92               | 92                          | 92                          | 92               | 2.5                         | 67 |
| 14                                            |                          | 12                         | 25              | 42               | 50               | 100                         | 100                         | 100              | 2.2                         | 50 |

* Groups of the three kinds of F1 recipients were injected intravenously on days 0 and 7 with a mixture of $5 \times 10^7$ viable B6 donor cells containing one-third lymph node cells and two-thirds spleen cells.

‡‡ 1 wk before the induction of the GVHR.

§§ All F1 mice from this group had died from acute GVHD by this time (Fig. 1).
time period between the induction of GVHR and death due to acute GVHD, none of the (bm1 × bm12)F1 recipients produced detectable amounts of autoantibodies or had elevated proteinuria (Table II). Thus, the B6 donor cells were incapable of inducing any symptoms of SLE-like GVHD in recipients differing at both I-A and K. Also, none of the (B6 × bm1)F1 recipients, which differed at the K locus alone, produced detectable amounts of autoantibodies early during the GVHR; at weeks 8 and 14 only, one-third of these GVH F1 mice had antibodies against nuclear antigens and 25% had antibodies against dsDNA (Table II). Furthermore, none of the GVH F1 mice of this group had antibodies against thymocytes in their serum or became Coombs' positive at any of the time points tested, and, significantly, none of them developed elevated proteinuria. These findings indicate that an incompatibility at K alone was insufficient to trigger a full-blown SLE-like syndrome, but, if at all, induced only mild symptoms of chronic GVHD.

By contrast, 2 wk after the initiation of GVHR the vast majority of the I-A-different (B6 × bm12)F1 recipients showed autoantibodies to thymocytes and nuclear antigens, and, during the course of the GVHR, all (B6 × bm12)F1 recipients had these autoantibodies at one or more of the times tested (Table II). IgG autoantibodies to dsDNA were detectable from week 4 onwards, and 67% of the (B6 × bm12)F1 recipients showed these antibodies at one or more times during the GVHR. Moreover, 42% of the GVH F1 mice were Coombs' positive at one or more of the times tested. Elevated proteinuria occurred in 50% of the GVH (B6 × bm12)F1 recipients. One-half of the mice with elevated proteinuria also developed ascites, indicating that these GVH F1 mice had a severe nephrotic syndrome. Those GVH (B6 × bm12)F1 recipients which had elevated proteinuria also showed immunohistological evidence of ICGN. Thus, incompatibility at I-A alone elicited a full-blown SLE-like GVHD.

B Cell Activity in the Spleens of the Three Kinds of GVH F1 Mice. As reported previously (2), nonirradiated, H-2-different F1 mice developing acute GVHD initially (week 1) showed a phase of strong B cell stimulation, which rapidly thereafter (week 2) reversed to severe B cell suppression. By contrast, F1 mice undergoing chronic GVHD showed a continuing stimulation of their B cells (2). These observations were made in groups of identical F1 mice that had been injected with genetically different T cells obtained from one of the two parental strains, C57BL/10 and DBA/2, respectively. Very similar results were made in the present study, where we injected genetically identical donor cells into groups of F1 recipients that carried different H-2 mutations.

Groups of (bm1 × bm12)F1, (B6 × bm1)F1, and (B6 × bm12)F1 mice were injected with 10⁶ B6 spleen cells. After 7 and 17 d, the spleen cells from the GVH F1 mice of these groups were assayed for the total number of IgG- and IgM-secreting cells. Groups of syngeneic N F1 mice served as controls. At 7 d after the initiation of the GVHR, the numbers of IgG- and IgM-secreting cells per 10⁶ spleen cells of the K- and I-A-different (bm1 × bm12)F1 recipients were increased by factors of ~5.5 and 2, respectively (Table III). In addition, the spleen weights of these GVH F1 animals were increased by a factor of 2.4 (Table IV). At 17 d after the initiation of the GVHR in such F1 mice, however, the numbers of IgG- and IgM-secreting spleen cells were decreased by a factor of 3–4 (Table III), although the spleen weights were still increased (Table IV). These findings indicate that induction of the GVHR in (bm1 × bm12)F1 recipients, which differed by mutations at both I-A and K, initially (week
Table III

Numbers of IgM- and IgG-secreting Cells in the Spleens of the Three Kinds of F1 Mice at Different Days after the Induction of GVHR

| Mice tested and H-2 difference indicated | Mean number (± SEM) of protein A PFC (× 10⁶) per 10⁶ nucleated spleen cells at indicated days after the induction of GVHR |
|-----------------------------------------|--------------------------------------------------------------------------------------------------|
|                                         | Day 7                                                                                          | Day 17                                                                 |
|                                         | IgM                          | IgG                          | IgM                          | IgG                          |
| (bml × bm12)F1                          | 1.6 ± 0.3§                    | 6.7 ± 2.5§                    | 0.2 ± 0.06‖                   | 0.3 ± 0.07‖                   |
| K + I-A (bml × bm12)F1                  | 0.8 ± 0.1                      | 1.2 ± 0.2                      | 0.6 ± 0.1                      | 1.2 ± 0.1                      |
| (B6 × bm12)F1                          | 0.9 ± 0.1§                     | 0.8 ± 0.1§                     | 0.8 ± 0.1§                     | 0.8 ± 0.2§                     |
| K (B6 × bm12)F1                         | 0.6 ± 0.1                      | 0.6 ± 0.2                      | 0.6 ± 0.2                      | 0.6 ± 0.1                      |
| (B6 × bm12)F1                          | 1.3 ± 0.8§                     | 5.5 ± 0.8§                     | 3.1 ± 0.4§                     | 5.2 ± 0.9§                     |
| I-A (B6 × bm12)F1                       | 0.8 ± 0.2                      | 1.0 ± 0.1                      | 0.9 ± 0.2                      | 0.9 ± 0.2                      |

* GVHR was induced by intravenous injection of 10⁸ viable B6 spleen cells on day 0. Each group consisted of five mice.
† Noninjected F1 mice. Each group consisted of five mice.
§ Significantly higher (P < 0.05) when compared with the values of the corresponding N F1 mice.
‖ Significantly lower (P < 0.05) when compared with the values of the corresponding N F1 mice.
¶ Not significantly different from the values of the corresponding N F1 mice.

Table IV

Spleen Weights of the Three Kinds of F1 Mice at Different Times after the Induction of GVHR

| Mice tested and H-2 difference indicated | Spleen weights of the F1 mice* at indicated time after the initiation of GVHR |
|-----------------------------------------|--------------------------------------------------------------------------------|
|                                         | mg                                                                             |
|                                         | Day 7                                                                 | Day 17                                                                 |
| (bml × bm12)F1                          | 305 ± 15¶                                                                  | 308 ± 12¶                                                                  |
| K + I-A (bml × bm12)F1                  | 128 ± 10                                                                     | 122 ± 4                                                                    |
| (B6 × bm12)F1                          | 115 ± 8**                                                                    | 202 ± 30**                                                                  |
| K (B6 × bm12)F1                         | 120 ± 9                                                                      | 125 ± 2                                                                    |
| (B6 × bm12)F1                          | 140 ± 10**                                                                   | 419 ± 25**                                                                  |
| I-A (B6 × bm12)F1                       | 117 ± 8                                                                      | 120 ± 24                                                                   |

* Five mice per group.
‡ GVHR was induced by intravenous injection of 10⁶ viable B6 spleen cells on day 0.
§ Noninjected F1 mice.
¶ Arithmetic mean ± SEM.
** Not significantly different (P > 0.05) from the values of the corresponding N F1 mice.

1) led to a strong B-cell stimulation which rapidly thereafter (week 3) changed into suppression.

At 7 d after the initiation of GVHR in (B6 × bm1)F1 mice, which differed by the K mutation only, the total numbers of IgG- and IgM-secreting cells per 10⁶ spleen cells were not significantly different from the numbers found in the spleens of the corresponding N F1 mice (Table III). The spleen weights of these GVH F1 mice were not significantly different from the spleen weights of N F1 mice (Table IV). At 17 d
after the initiation of the GVHR, the numbers of IgG- and IgM-secreting cells were per 10⁶ spleen cells slightly but not significantly increased. The mean spleen weight of these GVH F₁ animals was increased by a factor of 1.6 (Table IV). Thus, B6 donor cells induced neither a strong stimulation nor suppression of the B cells in the K-different (B6 × bm1)F₁ recipients, and only a marginal splenomegaly was induced in these F₁ recipients.

7 d after the induction of the GVHR in the I-A-different (B6 × bm12)F₁ mice, the number of IgG-secreting cells per 10⁶ nucleated spleen cells was increased by a factor of 5.5, and the total number of IgM-secreting cells by a factor of about 1.6 (Table III). At this point in time, the spleen weights of the GVH (B6 × bm12)F₁ mice were not different from those of the corresponding N F₁ mice (Table IV). 17 d after the initiation of the GVHR, the numbers of IgG- and IgM-secreting cells per 10⁶ spleen cells were increased by a factor of 5.7 and 3.4, respectively (Table III). At that time, the spleen weights of the (B6 × bm12)F₁ recipients were increased by a factor of 3.5 (Table IV), and the numbers of nucleated cells in the spleens were increased by a factor of 2 when compared with the numbers in the spleens of N F₁ mice. Therefore, the total numbers of IgG- and IgM-secreting cells in the spleens of (B6 × bm12)F₁ recipients were, in fact, increased by factors of 11 and 7. These findings indicate that B6 donor cells failed to suppress and exclusively stimulated the B cells of the I-A-different (B6 × bm12)F₁ recipients. Moreover, the number of IgG-secreting cells in the spleens of the GVH (B6 × bm12)F₁ mice, used for the induction of acute and chronic GVHD (Fig. 1; Tables I and II), was increased by a factor of 3 at time of autopsy (day 112 after the induction of the GVHR) (data not shown). This indicates

![Fig. 2](image)

**Fig. 2.** Addition of graded numbers of spleen cells obtained from GVH F₁ mice to SRBC-stimulated cultures of 3 × 10⁸ spleen cells from syngeneic N F₁ mice. The GVHR had been induced 10 d before by injecting into the three kinds of F₁ mice 10⁶ spleen cells from B6 donors. Anti-SRBC plaque-forming cells (PFC)/culture were determined on day 4 and plotted as percent of PFC of control cultures to which no GVH spleen cells had been added [(B6 × bm12)F₁: 100% = 740 PFC/culture (○); (B6 × bm1)F₁: 100% = 1460 PFC/culture (□); (bm1 × bm12)F₁: 100% = 762 PFC/culture (△)]. Each point represents the mean ± SD of four cultures. (○), spleen cells from GVH (B6 × bm12)F₁ mice added to spleen cells from normal (B6 × bm12)F₁ mice; (□), spleen cells from GVH (B6 × bm1)F₁ mice added to spleen cells from normal (B6 × bm1)F₁ mice; (△), spleen cells from GVH (bm1 × bm12)F₁ mice added to spleen cells from normal (bm1 × bm12)F₁ mice.
that B6 donor cells induced a persistent stimulation of IgG-secreting B cells in (B6 × bm12)F1 recipients that differed by the I-A mutation only.

The Activity of Alloreactive Donor T\(H\) and T\(S\) Cells in the Splenies of the Three Kinds of GVH F\(1\) Mice. It has been shown (2, 20) that spleen cells obtained from nonirradiated F\(1\) mice suffering from acute GVHD (weeks 2–4) suppress the primary anti-SRBC response of normal F\(1\) spleen cells in vitro. This suppression was due to F\(1\)-specific alloreactive donor T\(S\) cells (20, 23, 24), the activity of which peaked around day 10 after the initiation of the GVHR (2, 20). By contrast, spleen cells from GVH F\(1\) mice suffering from stimulatory GVHD failed to suppress the primary anti-SRBC response of normal F\(1\) spleen cells in vitro (2). To more precisely determine the alloantigens to which donor T\(H\) and T\(S\) cells, respectively, react, spleen cells obtained from the three kinds of mutant GVH F\(1\) mice were also tested in such an assay system.

As few as 6.4 \(\times 10^4\) spleen cells obtained from GVH (bm1 × bm12)F\(1\) mice almost completely suppressed the primary anti-SRBC response of 5 \(\times 10^6\) normal (bm1 × bm12)F\(1\) spleen cells (Fig. 2). Thus, a mutation in the F\(1\) recipients at both K and I-A had led to maximal activation of B6 allosuppressor T cells in week 2 after the induction of the GVHR. Spleen cells from GVH (B6 × bm1)F\(1\) mice, which differ at the K locus only, also suppressed the primary anti-SRBC of normal (B6 × bm1)F\(1\) spleen cells. This suppression, however, was far less profound when compared with the suppression induced by GVH (bm1 × bm12)F\(1\) spleen cells. Spleen cells from GVH (B6 × bm12)F\(1\) mice failed to suppress but helped the primary anti-SRBC response of normal (B6 × bm12)F\(1\) spleen cells. Thus, a mutational difference between donor and host at I-A alone appears to exclusively activate alloreactive donor T\(H\) cells.

Discussion

There is increasing evidence that functionally different subsets of donor T cells, which may act synergistically as well as antagonistically, are responsible for the wide spectrum of different pathological GVH symptoms (2–5, 8, 9, 20, 23, 25). Hence, in attempts to dissect the cellular mechanism underlying stimulatory and suppressive GVH symptoms, respectively, it is crucial that assays be used which reflect the different and sometimes even antagonistic functions of subsets of donor T cells. The spleen weight assay and the popliteal lymph node weight assay do not measure specific cellular function and preferentially reflect stimulatory rather than suppressive activities of donor T cells. The GVH mortality assay, too, is inadequate because, at least in nonirradiated recipients, it reflects the activity of alloreactive donor T\(S\) cells (4), but does not measure allohelp. Moreover, GVH mortality can greatly vary due to nonimmunological factors, such as the quality of the microflora in the intestine of the recipients (2, 26).

A GVH assay by which one can measure the activity of both alloreactive donor T\(H\) and T\(S\) cells has been described by Pickel and Hoffmann (20, 23). By using a parent \(\rightarrow\) F\(1\) hybrid combination differing at all of H-2, they showed that spleen cells obtained from GVH F\(1\) mice on day 3 after the initiation of GVHR exerted a positive allogeneic effect on the primary anti-SRBC response of N F\(1\) spleen cells in vitro; by contrast, spleen cells obtained on days 7–20 exerted a negative allogeneic effect (20, 23). Allohelp in this system was provided by F\(1\)-specific Lyt-1\(^+\)2\(^-\) donor T cells present in the spleens of the GVH F\(1\) mice (20, 23), whereas allosuppression was caused by F\(1\)-
Table V

Summary of the Presented Data

| GVHR F1 mice tested and H-2 difference indicated* | Number of Ig-secreting cells in the spleen at indicated time after the initiation of GVHR‡ | Effect of GVHR F1 spleen cells on the primary anti-SRBC response of N F1 spleen cells in vitro (day 10)§ | SLE-like autoantibodies and elevated proteinuria∥ | Clinical signs of acute GVHD and induction of LGVHD¶ |
|--------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-----------------------------------|-----------------------------------|
| (B6 × bm12)F1 | ↑ | ↑ | + | – |
| I-A | | | | |
| (B6 × bm1)F1 | 0 | 0 | 0/↓ | ± | – |
| K | | | | |
| (bm1 × bm12)F1 | ↑ | ↓ | ↓ | – | + |
| K + I-A | | | | |

* GVHR was induced by injection of B6 lymphocytes.
‡ ↑, increase; ↓, decrease.
§ 0, No increase or decrease; 1, increase; ↓, decrease.
∥ –, Absent; ±, weakly present or absent; +, present.
¶ –, Absent; +, present.

specific Lyt-1^+2^+ donor T cells (20, 23, 24). In a preliminary report from our laboratory, the results obtained by this assay system were found to correlate with the histopathological changes taking place in the lympho-hemopoietic tissue of H-2-different GVH F1 mice. It was shown that the activation of allosuppressor donor T cells preceded and coincided with the pancytopenia characteristic of acute GVHD (2). In addition, the protein-A PFC assay (19) was used as an indicator of total B cell activity in the spleens of the GVH F1 mice. This assay, too, clearly reflected the sequential activation of first allohelper and then allosuppressor donor T cells during the first 3 wk after the initiation of the GVHR (2).

In the present investigation, the results obtained in H-2-mutant GVH F1 mice by means of the Pickel and Hoffmann assay and the protein-A PFC assay were compared with those obtained by more conventional GVH assays. A mutational difference, in the F1 recipient, solely at I-A was shown to activate exclusively allohelper T cells of the B6 donor and led to a continuous production of stimulatory GVH symptoms. By contrast, a mutational difference at both I-A and K led to the sequential activation of B6 allohelper and allosuppressor T cells. This rapid sequence of differential alloactivation in vivo was closely paralleled by stimulatory and suppressive GVH symptoms, respectively, as measured by more conventional GVH assays. A mutational difference at K alone, however, failed to optimally activate either the allohelper or the allosuppressor T cells of the B6 donor. The results of our studies are summarized in Table V.

Induction of an SLE-like Stimulatory GVHD across an I-A Mutation. The bm12 mutant is of the gain and loss type (27), maps to the I-A subregion (18), and consists of but a limited number of peptide differences in the Aβ polypeptide when compared with B6 (28). Functional studies have shown that the mutation resulted in strong reciprocal mixed lymphocyte reactions (18) and the generation of cytotoxic T cells (29) between B6 and bm12. By studying the genetic requirements for the induction of abnormal T-B cell cooperation in vitro, we found that B6 T cells can optimally help (positive allogeneic effect), but cannot suppress (negative allogeneic effect) bm12 B cells in a
primary anti-SRBC response.\textsuperscript{4} Hence, it was concluded that, in vitro, the I-A mutation difference triggers alloreactive T\textsubscript{H} cells and no, or almost no, alloreactive T\textsubscript{S} cells.\textsuperscript{4}

The data presented here indicate that an I-A incompatibility in vivo preferentially triggers the donor T cells responsible for the induction of SLE-like GVHD (Table V); these T cells are the alloreactive T\textsubscript{H} cells (8, 9) possessing the Lyt-1\textsuperscript{+}2\textsuperscript{−} phenotype.\textsuperscript{3} Thus, both in vitro\textsuperscript{4} and in vivo, an I-A difference triggers alloreactive donor T\textsubscript{H} cells and no, or almost no, alloreactive donor T\textsubscript{S} cells. In the latter aspect, the I-A incompatibility differs from an incompatibility at both I-A and K, which causes a profound allosuppression both in vitro\textsuperscript{4} and in vivo (Table V).

In a previous paper (9), we showed that an I-E difference between the parental donor and F\textsubscript{1} host led to the induction of SLE-like GVHD. In vitro, the same I-E difference induced allohelp but no allosuppression.\textsuperscript{4} Thus, both in vitro and in vivo (at least in nonirradiated F\textsubscript{1} recipients), class II alloantigens (I-A and I-E) trigger mainly alloreactive T\textsubscript{H} cells. It should be noted, however, that Swain and Dutton (30) were able to measure allosuppression across class II differences only; but, as opposed to our test system in vitro\textsuperscript{4} and that of Cantor and Boyse (16), Swain and Dutton used a population of primed spleen cells to which they added T cells differing at class II alloantigens (30). Furthermore, Klein and Chiang (15), who used F\textsubscript{1} recipient mice that had been irradiated before the induction of GVHR, were able to induce LGVHD across class II differences only. It is unlikely though that LGVHD in that system was caused by alloreactive donor T\textsubscript{H} cells, because Lyt-1\textsuperscript{+}2\textsuperscript{−} donor cells were found to be incapable of inducing LGVHD in irradiated F\textsubscript{1} recipients across a total H-2 difference.\textsuperscript{3} One possible explanation for the discrepancy in the outcome of the GVHR in irradiated and nonirradiated F\textsubscript{1} mice differing at class II alloantigens is the difference in susceptibility to LGVHD in both kinds of F\textsubscript{1} recipients. Irradiated recipients are much more sensitive than nonirradiated ones for the induction of LGVHD (31). Therefore, it is possible that a low amount of allosuppression, which is ineffective in nonirradiated F\textsubscript{1} recipients, is effective in inducing LGVHD in irradiated F\textsubscript{1} recipients.

\textit{Neither Acute nor Stimulatory GVHD Were Induced across a Mutational Difference at the K Locus Alone.} Lymphocytes from bm1 and B6 produce a positive mixed lymphocyte reaction in both directions (32), and bm1-anti-B6 and B6-anti-bm1 cytotoxic T cells can also be generated (33, 34). Biochemical studies showed that the H-2K\textsuperscript{b} product of the bm1 mutant differs from the product of B6 by only two amino-acid substitutions (35).

In the present study, the GVHR across this small H-2K\textsuperscript{b} mutational difference was studied. Neither acute GVHD nor a full-blown SLE-like GVHD could be induced in the (B6 × bm1)F\textsubscript{1} mice by injection of the B6 donor cells (Table V). Similar results were obtained with combinations involving intra-H-2-recombinant strains that differed only at K or D (unpublished results). These findings indicate that a solely class I (K mutation) difference between donor and host cannot optimally trigger the T cells responsible for the induction of stimulatory GVHD or the T cells that induce acute GVHD. A possible explanation for this might be the relatively low amount of allohelp triggered by the H-2K\textsuperscript{b} mutation. Evidence from three different experimental systems clearly indicates that the induction of both acute and stimulatory GVHD in nonirradiated F\textsubscript{1} mice requires strong allohelp (2, 20).\textsuperscript{3} Although we\textsuperscript{4} and others (36, 37) showed that it is possible to generate allohelp as well as allosuppression across this
H-2K^b mutation in vitro, the amount of allohelp was low when compared with the amount triggered by class II molecules. The lack of distinct stimulatory and suppressive GVH symptoms triggered by the H-2K^b mutation cannot be ascribed to a numerical deficit in the corresponding alloreactive T cells. Swain et al. (36) reported that the precursor frequency of alloreactive T cells that can be triggered by the K mutation difference of bm1, is only a factor ~2 lower than the frequency of alloreactive T cells triggered by a total H-2 difference. Nevertheless, we found that increasing the number of donor cells (1.6 × 10^6 instead of 1 × 10^6 B6 cells) injected into the (B6 x bm1)F1 mice still failed to induce clear-cut stimulatory or suppressive GVHD (data not shown). Alternatively, certain qualitative differences between the allohelper T cells triggered by class I (K mutant) and class II (I-A mutant) might account for the observed differences in GVHD elicited by the I-A or I-A plus K mutational differences on the one hand and the K mutation alone on the other (Table V).

**Induction of Acute GVHD across a K Plus I-A Difference.** Our findings show that a mutational difference at both I-A and K, but neither at I-A nor K alone, triggers the donor T cells capable of inducing acute GVHD (Table V). In previous papers (2-5), from our laboratory, evidence has been provided that acute GVHD is due to the sequential activation of first alloreactive donor TH and eventually donor Ts cells. The data presented here strongly support this concept. The stimulatory symptoms observed in the (bm1 x bm12)F1 mice early after the injection of B6 donor cells can be ascribed to alloreactive donor T H cells activated by the I-A mutation, whereas the ensuing suppressive symptoms must have been caused by alloreactive donor Ts cells. Since no suppressive symptoms characteristic of acute GVHD could be induced across a K mutation difference alone, i.e., in the combination B6 → (B6 x bm1)F1, obviously the alloreactive donor T H cells, triggered by the I-A incompatibility, were needed for the activation of the alloreactive donor Ts cells. Thus, two signals are required for the optimal induction of donor allosuppressor T cells in vivo: a class II difference (I-A mutation) that elicits maximal allohelp, and the additional existence of a class I difference (K mutation) to which the donor Ts cells react. This requirement of both a specific antigenic difference (class I) and maximal help elicited by a class II difference is identical with the requirements for the optimal activation of cytotoxic T cells in vitro (38, 39). Furthermore, the need of activated T H cells for the differentiation from Ts precursor to Ts effector cells follows the general rules of the T cell circuit formulated by Cantor and Gershon (40). Apparently, this sequence of events is also required for the induction of the allosuppressive effects characteristic of acute GVHD.

The small antigenic difference between GVH (B6 x bm12)F1 and GVH (bm1 x bm12)F1 mice deserves comment. Although this difference was restricted to but two amino acids in the K molecule (35), it caused an extraordinary difference in pathological symptoms (Table V). Within the spectrum of GVH symptoms (3), these two GVH combinations occupy exactly opposite positions, in that the former combination developed the stimulatory symptoms of SLE-like GVHD, whereas the latter developed the pancytopenia characteristic of acute GVHD. As we shall discuss below, this finding might be able to improve our understanding of the association of certain immunological diseases with structures of the major histocompatibility complex in man, HLA.
Comparison with Etiologic Agents Causing GVHR-like Pathological Changes. Essentially the same spectrum of stimulatory and suppressive symptoms as that induced by the GVHR has been observed in patients exposed to sensitizing drugs or undergoing viral infections (3). For instance, some of the patients sensitized to the drug diphenylhydantoin show hypergammaglobulinemia and SLE-like autoantibodies, whereas others, sensitized to the same drug, show hypogammaglobulinemia and/or aplastic anemia. A similar GVH-like spectrum of different symptoms has been observed in patients infected with Epstein-Barr virus (3). In view of this similarity of the pathological symptoms, it has been postulated that the basic cellular mechanism underlying these diseases might consist of GVH-like reactions of T cells to autologous lympho-hemopoietic cells or macrophages rendered foreign by the drug or the virus (3). Evidence has been obtained from studies in mice that indeed diphenylhydantoin attaches to the surface of lymphoid cells and then triggers GVH-like reactions by autologous T cells (41, 42). The GVHR triggered by small mutational changes in the primary structure of class I and/or class II alloantigens, as studied in the present investigation, closely resembles this situation of “altered self.” Conceivably, the combined recognition by autologous T cells of self-major histocompatibility complex (MHC) structures and the respective etiologic agent might cause the GVH-like diseases observed in individuals undergoing viral infections or exposed to sensitizing drugs. Furthermore, our data indicate that possible somatic mutations that affect the MHC structures of lymphoid cells might also lead to GVH-like diseases.

As has been discussed in detail elsewhere (9), products of the same locus, HLA-DR, appear to be involved in the pathogenesis of SLE, irrespective of whether the disease was idiopathic or induced by a drug. Because HLA-DR is a functional analogue of the murine I-A/I-E loci (43), it is tempting to speculate that the cellular mechanism responsible for the induction of SLE in humans is the same as the mechanism responsible for the induction of SLE-like GVHD in mice (8, 9). By analogy, the spontaneous development of suppressive GVH-like symptoms in patients might be due to the combined recognition by autologous T cells of the etiologic agent and class I as well as class II MHC structures. It is interesting, therefore, that in patients taking diphenylhydantoin a low serum IgA level was associated with an increased frequency of HLA-A2 (44). Whether there also exists a correlation with HLA-DR in such patients is not currently known.

Summary

By induction of a graft-vs.-host reaction (GVHR) in nonirradiated H-2-different F₁ mice, one can induce stimulatory pathological symptoms, such as lymphadenopathy and hypergammaglobulinemia, combined with the production of autoantibodies characteristic of systemic lupus erythematosus (SLE). Alternatively, the GVHR can lead to the suppressive pathological symptoms, such as pancytopenia and hypogammaglobulinemia, characteristic of acute GVH disease (GVHD). Whether stimulatory or suppressive symptoms are induced by a GVHR depends, in our view (2-4), on the functional subset of donor T cells activated in the F₁ host. The purpose of the present study was to investigate whether class I and/or class II H-2 alloantigens can selectively trigger, out of a pool of unselected donor T cells, those subpopulations of T cells responsible for the stimulatory and suppressive GVH symptoms, respectively.

For the induction of the GVHR, 10⁹ lymphoid cells from C57BL/6 (B6) donors
were injected into three kinds of F1 hybrid mice, which had been bred from H-2 mutant strains on a B6 background. Whereas the I-A-disparate (B6 × bm12)F1 recipients exclusively developed stimulatory GVH symptoms, including SLE-like autoantibodies and immune complex glomerulonephritis, the K locus-disparate (B6 × bm1)F1 recipients showed neither clearly stimulatory nor clearly suppressive GVH symptoms. In marked contrast, the (bm1 × bm12)F1 recipients, which differ from the B6 donor strain by mutations at both K and I-A locus, initially developed stimulatory GVH symptoms, but rapidly thereafter showed the suppressive pathological symptoms of acute GVHD and died. Moreover, spleen cells obtained from (B6 × bm12)F1 mice injected with B6 donor cells helped the primary anti-sheep erythrocyte (SRBC) response of normal (B6 × bm12)F1 spleen cells in vitro, whereas spleen cells (bm1 × bm12)F1 mice injected with B6 donor cells strongly suppressed the primary anti-SRBC response of normal (bm1 × bm12)F1 spleen cells. Spleen cells from the K locus-disparate (B6 × bm1)F1 recipients also suppressed the primary anti-SRBC of normal (B6 × bm1)F1 spleen cells; this suppression, however, was weak when compared with the suppression induced by spleen cells from GVH (bm1 × bm12)F1 mice.

Taken together, these findings indicate that a small class II (I-A) antigenic difference suffices to trigger the alloreactive donor T helper cells causing SLE-like GVHD. In contrast, both class I (H-2K) and class II (I-A) differences are required to trigger the subsets of donor T cells responsible for acute GVHD. It appears that alloreactive donor T helper cells induce the alloreactive T suppressor cells, which then act as the suppressor effector cells causing the pancytopenia of acute GVHD. These findings may help to understand the variability of GVH-like diseases caused by a given etiologic agent, their cellular pathogenesis, and association with certain HLA loci.

We are obliged to Dr. C. J. M. Melief for his gift of the mutant mice B6.C.H-2 bm1 and B6.C.H-2 bm12. The technical assistance of Walter van der Meer, Deon Kanters, and Nellie de Boer-Bakker is gratefully acknowledged. Our colleagues Drs. L. A. Aarden, P. Ivanyi, C. J. Lucas, and C. J. M. Melief are thanked for their critical reading of the manuscript.

Received for publication 23 September 1982.

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