PROTECTION AGAINST GRAFT VS. HOST-ASSOCIATED IMMUNOSUPPRESSION IN F1 MICE

I. Activation of F1 Regulatory Cells by Host-specific Anti-Major Histocompatibility Complex Antibodies

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Inoculation of immunocompetent lymphoid cells into allogeneic or semisyngeneic F1 recipient mice can lead to graft vs. host (GvH) reactions (1-3). In the induction phase of the GvH the injected lymphocytes recognize and respond to foreign alloantigens expressed by recipient cells (4). Such recognition can result in the generation of a cytotoxic effector T lymphocyte (CTL) response against the host cells that may be associated with the characteristic symptoms of GvH disease (4-6). Other facets of GvH reactivity are the production of autoantibodies and the development of severe immunoincompetence (7). It has also been shown that the injection of parental spleen cells into unirradiated F1 hybrid mice results in reduction or abrogation of the capability to generate a cytotoxic T cell response in vitro (8). This lack of immune responsiveness could be accounted for by at least two possible suppressive mechanisms: (a) activation of parent-anti-F1 allogeneic CTL; and (b) activation of a noncytotoxic F1 suppressor population resulting from parent-anti-F1 recognition (9).

Earlier reports indicated that GvH reactivity as detected by splenomegaly and mortality could be prevented or reduced by injecting F1 mice with alloantisera (10, 11). In the present study we have attempted to protect F1 hybrid mice from the induction of GvH-associated suppression of the CTL response by injecting F1 mice with alloantibodies before inoculation of parental spleen cells. The results described here indicate that alloantiserum directed against either parental haplotype of the F1 as well as certain monoclonal antibodies against H-2 or Ia antigens of the F1 host prevented the induction of GvH-associated CTL suppression. Moreover, adoptive transfer studies showed that such protection is due to a radiosensitive F1 regulatory T cell population, which is activated by specific alloantibodies.

Materials and Methods

Mice. Male mice, 8-12 wk of age, were used throughout these studies. All mice used [(C57BL/10 × B10.A)F1] [(B10 × B10.A)F1], (C57BL/10 × B10.BR)F1 [(B10 × BR)F1], B10.A, and B10.BR (BR)] were obtained from The Jackson Laboratory, Bar Harbor, Maine.

Antibodies. The alloantisera used in these experiments are described in Table I. The

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Abbreviations used in this paper: CTL, cytotoxic T lymphocytes; GvH, graft vs. host; HBSS, Hanks' balanced salt solution; MHC, major histocompatibility complex; NMS, normal mouse serum; PBS, phosphate-buffered saline.

1922 Journal of Experimental Medicine · Volume 1154, December 1981 · 1922-1934
monoclonal antibodies were used in ascites fluid form. The characteristics of the monoclonal reagents have been described in detail elsewhere (12). Some of their properties are summarized in Table II.

**Injection of F1 Hybrid Mice with Antibodies and Parental Spleen Cells.** The titers of alloantisera and monoclonal reagents were adjusted to the same concentration of cytotoxic antibodies by diluting with phosphate-buffered saline (PBS). The reagents were injected intravenously via the tail vein into normal F1 recipients in a volume of 0.1 ml or 0.5 ml. 24 h later, half of the mice from each group were injected intravenously with parental spleen cells suspended in a volume of 0.5 ml in Hanks' balanced salt solution (HBSS) (8).

**Treatment with a-Thy-1.2 Antibodies Plus Complement.** T cells were eliminated by incubation of 100 × 10⁶ spleen cells in 1 ml monoclonal anti-Thy-1.2 reagent (New England Nuclear, Boston, Mass.) (dilution 1:100) for 30 min at 37°C. After washing the cells in HBSS once, the spleen cells were then incubated with selected rabbit complement for 30 min at 37°C. The treated cells were washed twice and readjusted to the desired cell concentration.

**In Vitro Generation of and Assay for Cell-mediated Lympholysis.** The potential of the treated F1 hybrid mice to generate cytotoxic T cells in vitro was tested 8-14 d after inoculation of the parental spleen cells. In most cases, spleen cells from two mice per group were pooled. The responding cells were sensitized against 2,000-rad irradiated allogeneic stimulators for 5 d. The effector cells were tested in a 4-h ⁵¹Cr release assay on concanavalin A-stimulated splenic blasts. These conditions for sensitization and assay have been described previously (13). The percent lysis is expressed above medium control. Standard errors of the mean were usually <3% and have been excluded from the graphs for simplicity.

**Results**

**Abrogation of Parental Spleen Cell Induced Immunosuppression by Specific Alloantisera.** In an attempt to protect F1 mice from parental-induced GvH-associated immunosuppression (8), (B10 × B10.A)F1 animals were injected with anti-H-2² serum before inoculation with B10.A parental spleen cells. 24 h later half of the mice injected with the antibodies were inoculated with 15 × 10⁶ B10.A spleen cells. After a period of 8 d, the immune potential of injected F1 mice was tested. F1 lymphocytes were stimulated in vitro against BR allogeneic spleen cells, and their cytotoxic T cell activity was determined 5 d later.

**Table I**

| Product number | Antiserum | Immunization | Titer | Target cells |
|----------------|-----------|--------------|-------|--------------|
| 1366           | Anti-H-2² | B10-anti-B10.A | 1:64  | B10.A spleen cells |
| 1364           | Anti-H-2² | B10.A-anti-B10 | 1:32  | B10 spleen cells |
| 979            | Anti-H-2K²-1a² | (B10xA/J)f1 - anti-B10.D2 | 1:129 | B10.D2 spleen cells |
|                |           |              | <1:2  | B10.A spleen cells |

**Table II**

| Code          | Product number | Specificity | Titer   | Target cells |
|---------------|----------------|-------------|---------|--------------|
| 16-1-11N      | 5164           | Anti-K      | 1:2,000 | C3H spleen cells |
| 14-4-4S       | 5601           | Anti-I-E    | 1:32,000 | C3H spleen cells |
| 13-5-3S       | 5210           | Anti-D      | 1:4,000 | C3H spleen cells |
| 34-2-12S*     | 5888           | Anti-D      | 1:32,000 | B10.A spleen cells |

* Ozato, K., D. H. Sachs, and N. Mayer. Manuscript in preparation.
The data of Fig. 1 show the allogeneic CTL response of (B10 × B10.A)F1 spleen cells from mice treated with 0.1 ml anti-H-2α alloantiserum (cytotoxic titer 1:64), and subsequently inoculated with B10.A parental spleen cells. The cytotoxic activity of the effectors from the antiserum-treated mice was compared with the response of untreated F1 animals or of mice injected with normal mouse serum (NMS). The results indicate that pretreatment of the F1 mice with anti-H-2α alloantiserum before inoculation of the parental lymphocytes resulted in an allogeneic cytotoxic response (Fig. 1 F) almost equivalent to that of normal mice (Fig. 1 A). In contrast, the CTL potential of spleen cells from F1 mice that received no antiserum or NMS before injection with the same number of NMS parental spleen cells was strongly suppressed (Fig. 1 B, D).

Since the anti-H-2α antiserum could have acted on host cells, donor cells, or both, it was important to determine whether antiserum that is specific for the host only would protect against GvH-induced suppression. Therefore, the effect of anti-H-2β

![Graphs showing cell-mediated cytotoxicity](image-url)

**Fig. 1.** Cell-mediated cytotoxicity of (B10 × B10.A)F1 spleen cells from F1 mice that were (A) untreated, or injected with (B) 15 × 10⁶ B10.A spleen cells; (C) 0.1 ml NMS i.v.; (D) NMS and after 24 h with B10.A spleen cells intravenously; (E) 0.1 ml anti-H-2α serum; (F) anti-H-2α serum and after 24 h with B10.A spleen cells intravenously; (G) 0.1 ml anti-H-2α serum; or (H) anti-H-2α serum and after 24 h with B10.A spleen cells. Effector cells were generated by in vitro sensitization against BR spleen cells, 8 d post inoculation of B10.A spleen cells. (○) Effectors stimulated in vitro against BR spleen cells and tested on BR blasts; (□) effectors cultured without stimulators and tested on BR blasts (background stimulation).
alloantiserum on parental spleen cell-induced suppression was tested. (B10 × B10.A)F1 mice were treated according to the protocol described above. Fig. 1H shows that suppression of the allogeneic CTL response was likewise reduced if F1 mice were injected with 0.1 ml of anti-H-2b alloantiserum (cytotoxic titer 1:32) before inoculation of the B10.A parental spleen cells. Thus, these data indicate that alloantibodies directed against either parental haplotype of the F1 reduced parental spleen cell induced immunosuppression.

One possible explanation for the reduction of immunosuppression in the antibody-treated mice could be that circulating alloantibodies in the F1 host were absorbed by the injected B10.A spleen cells and that the parental lymphocytes were eliminated. Therefore, the serum of F1 mice was assayed 24 h after injection of 0.1 ml of undiluted antiserum for complement-dependent cytotoxicity. Table III shows that serum from F1 mice previously treated with anti-H-2a or anti-H-2b alloantiserum did not exhibit any detectable cytotoxicity on the specific target cells. In contrast, serum from B10 or B10.A mice injected with the same amount of nonspecific alloantiserum still contained circulating antibodies with cytotoxic activity when tested 24 h later.

Specificity of the antibodies was tested by injecting alloantiserum that did not recognize antigens expressed by either parental haplotype in the F1. As demonstrated in Fig. 2, pretreatment of (B10 × B10.A)F1 mice with an irrelevant alloantiserum (anti-H-2Kd, Ia^d) before the injection of B10.A parental spleen cells had no effect on the suppression of the allogeneic CTL response (Fig. 2F). These data indicate that specific antibodies against either haplotype of the F1 strain, but not antibodies specific for antigens not expressed by the F1 host, are capable of reducing the parental spleen cell-induced immunosuppression.

To determine the amount of alloantibodies required to abrogate B10.A parental spleen cell-induced suppression in the (B10 × B10.A)F1 recipients, mice were injected

### Table III

*Complement-dependent Cytotoxicity of Serum from Mice Injected with 0.1 ml Alloantiserum 24 h Previously*

| Serum/dilutions         | Normal (B10 × B10.A)F1 | F1 injected with NMS | F1 injected with anti-H-2^a^ | F1 injected with anti-H-2^b^ | Anti-H-2^a^ | Anti-H-2^b^ | B10 injected with anti-H-2^a^ | B10 injected with anti-H-2^b^ | B10.A injected with anti-H-2^a^ | B10.A injected with anti-H-2^b^ |
|-------------------------|-------------------------|----------------------|-------------------------------|-------------------------------|-------------|-------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Percent trypan blue positive cells | 1:2         | 1:4                  | 1:2                          | 1:4                          | 11          | 72          | 11                            | 13                            | 46                            | 63                            |

* Complement control 11%.
‡ Complement control 14%.
PROTECTION AGAINST GRAFT VS. HOST BY ANTISERA

EFFECTORS: (B10×B10.A)F1 spleen cells

EFFECTORS: F1 spleen cells

EFFECTORS: B10.A spleen cells

EFFECTOR: TARGET CELL RATIO

Fig. 2. Cell-mediated cytotoxicity of (B10 × B10.A)F1 spleen cells from F1 mice which were (A) untreated, or injected with (B) 15 × 10⁶ B10.A spleen cells; (C) 0.1 ml anti-H-2a serum; (D) anti-H-2a serum and B10.A spleen cells 24 h later; (E) 0.1 ml anti-H-2Kd-Iaα serum; (F) anti-H-2Kd-Iaα serum and B10.A spleen cells 24 h later. Effector cells were generated 8 d after injection of the B10.A spleen cells and assayed as described in legend of Fig. 1.

with various dilutions of anti-H-2a alloantiserum. The undiluted antiserum (cytotoxic titer 1:64) was diluted in PBS to 1:4 or 1:8, and 0.1 ml of each dilution was injected intravenously, followed by 15 × 10⁶ B10.A spleen cells 24 h later. 8 d post-inoculation of the parental cells, the F1 lymphocytes were sensitized in vitro against allogeneic BR stimulators. The results shown in Fig. 3 demonstrate that CTL suppression could be abrogated using a 1:4 dilution of the anti-H-2a alloantiserum diluted to a cytotoxic titer of 1:16 (Fig. 3 C). The data thus suggest that low amounts of alloantibodies provide protection from parental spleen cell-induced suppression. However, treatment with a further twofold dilution of the antiserum to 1:8 had no effect on the induction of CTL suppression in the F1 recipients (Fig. 3 D).

Treatment of the F1 Mice with Monoclonal Antibodies against H-2 Subregion Determinants. In an attempt to map the protective effect of the specific alloantisera, (B10 × B10.A)F1 mice were injected with monoclonal antibodies against different H-2 and Ia determinants. The various monoclonal reagents were adjusted to the same titer (1:25) and 0.5 ml of the diluted reagents was injected into each of the F1 mice 24 h before the injection of 15 × 10⁶ B10.A spleen cells. The potential for generation of alloreactive CTL in vitro was tested 8 d later. The data, summarized in Fig. 4, demonstrate that monoclonal reagents binding to Kk antigens (Fig. 4 B) or to I-Ek antigens (Fig. 4 C) completely abrogated B10.A spleen cell-induced suppression. In contrast, a monoclonal reagent binding to Dk antigens, an irrelevant antibody for the B10.A strain (Fig. 4 D), was ineffective.

Using the same monoclonal reagents, the effect of GvH-associated immune suppression was also tested in the (B10 × BR)F1 strain. The protocol for injection of the
Fig. 3. Cell-mediated cytotoxicity of (B10 × B10.A)F1 effector cells to BR alloantigen. Injection of F1 mice with various dilutions of anti-H-2\(^a\) alloantiserum. F1 mice were: (A) (○) untreated, (●) injected with 15 × 10\(^6\) B10.A spleen cells; (B) (○) injected with 0.1 ml undiluted anti-H-2\(^a\) alloantiserum, (●) undiluted anti-H-2\(^a\) serum plus 15 × 10\(^6\) B10.A spleen cells 24 h later; (C) (○) injected with 0.1 ml anti-H-2\(^a\) serum, 1:4 diluted, (●) anti-H-2\(^a\) serum 1:4 diluted plus 15 × 10\(^6\) B10.A spleen 24 h later; (D) (○) injected with 0.1 ml anti-H-2\(^a\) serum 1:8 diluted, (●) injected with anti-H-2\(^a\) serum 1:8 diluted plus 15 × 10\(^6\) B10.A spleen cells 24 h later. 8 d after injection of the B10.A spleen cells effector cells were generated and assayed as described in legend of Fig. 1. Solid lines indicate effectors cultured against BR spleen cells and tested on BR blasts; broken lines indicate effectors cultured without stimulators and tested on BR blasts.

Fig. 4. Cell mediated cytotoxicity of spleen cells from (B10 × B10.A)F1 mice injected with 0.5 ml of diluted monoclonal antibodies (cytotoxic titer adjusted to 1:25) to BR alloantigen. Spleen cells derived from F1 mice which were (A) (○) untreated, or injected with: (●) 15 × 10\(^6\) B10.A spleen cells; (B) (○) monoclonal anti-K\(^k\), (●) anti-K\(^k\) and B10.A spleen cells 24 h later; (C) (○) monoclonal anti-I-E\(^k\), (●) anti-I-E\(^k\) and B10.A spleen cells; (D) (○) monoclonal anti-D\(^k\), (●) anti-D\(^k\) and B10.A spleen cells. 8 d post-inoculation of B10.A spleen cells effector cells were generated and assayed as described in legend of Fig. 1. Solid lines indicate effectors stimulated against BR spleen cells and tested on BR blasts; broken lines indicate effectors cultured without stimulators and tested on BR blasts.
antibodies was identical to that used above. 9 d after inoculation of $20 \times 10^6$ BR parental spleen cells the generation of cytotoxic T cells against B10.A alloantigen was tested in vivo. Results similar to those observed in the (B10 × B10.A)F1 strain were obtained in the (B10 × BR)F1 mice, when treated with the anti-K\textsuperscript{b} or anti-I-E\textsuperscript{k} monoclonal reagents (Fig. 5). In addition, injection of monoclonal antibodies against D\textsuperscript{b} antigens prior to inoculation of BR parental spleen cells also diminished CTL immune suppression (Fig. 5 D). It should be noted that this anti-D\textsuperscript{k} reagent did not protect (B10 × B10.A)F1 mice from suppression (Fig. 4 E), which illustrates the specificity of these reagents in protecting against CTL suppression. Monoclonal anti-D\textsuperscript{d} antibodies, which are not reactive with either F1 haplotype in the (B10 × BR)F1, provided no protection from suppression (Fig. 5 E), although this reagent did protect against parental suppression in the (B10 × B10.A)F1 (data not shown). These data indicate that specific antibodies directed against H-2 determinants of either haplotype protect the F1 recipients against parental spleen cell induced suppression. However, this protective effect may not necessarily be limited only to anti-MHC antibodies.

**Activation of F1 Regulatory Cells by Anti-H-2\textsuperscript{a} Antiserum.** Among several possible explanations, protection against GvH-associated immunosuppression could be due to a regulatory process that involves the stimulation of a lymphoid cell population. If antibodies activate such regulatory cells, it should be possible to protect F1 mice by syngeneic lymphocytes adoptively transferred from alloantibody-treated animals. To test this, (B10 × B10.A)F1 mice were inoculated with $40 \times 10^6$ spleen cells from syngeneic F1 donor mice injected with 0.1 ml anti-H-2\textsuperscript{a} alloantiserum 24 h previously. The same recipients were also inoculated intravenously with $10 \times 10^6$ B10.A parental spleen cells and tested on B10.A blasts; broken lines indicate effectors cultured without stimulators and tested on B10.A blasts.

![Fig. 5](image-url)
U. HURTENBACH, D. H. SACHS, AND G. M. SHEARER

1929

spleen cells, which would normally be suppressive. The potential to generate a CTL response against BR alloantigen was tested 9 d later. Fig. 6C shows that the alloreactivity of spleen cells from these mice was comparable to the response of the untreated controls (Fig. 6A). In contrast, adoptive transfer of F1 spleen cells from untreated F1 donor mice, as shown in Fig. 6D, did not protect against CTL suppression (Fig. 6B). Thus, these data demonstrate that a regulatory cell population in the F1 host is activated by the alloantibodies that protect from GvH-associated CTL suppression.

To partially characterize this regulatory cell population, similar adoptive transfer studies were performed with irradiated or T cell-depleted spleen cells from syngeneic F1 mice. These F1 spleen cell donor mice were injected with 0.5 ml diluted monoclonal anti-K\textsuperscript{k} antibodies 24 h before adoptive transfer. Thus, (B10 X B10.A)F1 recipients were inoculated with 50 x 10\textsuperscript{6} F1 spleen cells (from antibody-treated donors) either treated with anti-Thy-1.2 plus complement or irradiated with 2,000 rad in addition to 10 x 10\textsuperscript{6} B10.A parental lymphocytes. 8 d later the CTL potential of spleen cells from these recipients was compared with mice inoculated with untreated F1 spleen cells from anti-K\textsuperscript{k} injected donors. As shown in Fig. 7, the protective effect of anti-K\textsuperscript{k}-activated F1 cells was completely eliminated after T cell depletion or irradiation with 2,000 rad. Thus, the anti-H-2-activated F1 cells that counteract CTL suppression are radiosensitive T lymphocytes.

Discussion

Previous studies have shown that unirradiated F1 hybrid mice injected with parental spleen cells develop some characteristics of GvH reactivity associated with nonspecific
suppression of the potential to generate a CTL response in vitro (8, 9). In the present study we have attempted to protect the F₁ recipients from CTL suppression by injecting mice with specific alloantibodies before inoculation of the parental spleen cells. The main findings of this study demonstrate that (a) F₁ mice can be protected from CTL suppression by alloantiserum directed against either parental haplotype; (b) protection can be achieved equally well with monoclonal reagents against H-2K, D, or I-E antigens; (c) protection is only provided by specific antibodies; (d) low titers of alloantibodies are sufficient to exert protection; and (e) protection can be adoptively transferred by radiosensitive, Thy-1-positive spleen cells from syngeneic donors previously treated with specific alloantiserum.

It has been previously reported that GvH disease can be decreased either by injecting the recipients (10, 11, 14, 15) or by treating the donor cells with antirecipient alloantibodies (16, 17). However, the mechanism of abrogation of GvH reactivity as assessed in those studies by splenomegaly and mortality rates is conjectural. Several explanations could account for the alloantibody-induced protection against immunosuppression. The first, covering of host major histocompatibility complex (MHC) determinants by the antibodies, which prevents their recognition of the inoculated parental effector cells, has been suggested previously (10). Such a mechanism can only account for protection by alloantibodies directed against H-2 determinants of the F₁ strain that are foreign to the inoculated parental cells. This does not explain protection by alloantibodies specific for the same haplotype of the parental inoculum, unless steric hindrance of the foreign allodeterminants is considered. Furthermore, the amount of antibodies injected is probably too small to saturate all alloantigenic sites in the F₁ host. A second explanation, binding of antibodies to donor MHC and thereby eliminating the relevant parental lymphocytes in vivo by complement-dependent lysis with the injected alloantibodies, is also unlikely. No evidence of
circulating antibodies was obtained 24 h after serum injection which could be absorbed by the inoculated parental spleen cells.

Anti-idiotype antibodies have been reported to inhibit GvH proliferation (18) and GvH disease (19). Although the presence of anti-idiotype antibodies in the anti-MHC alloantisera or in the monoclonal reagents derived from ascites fluid is theoretically possible (20), this explanation seems unlikely, because the protocol for generation of anti-idiotypic antibodies is different from that used to raise these alloantibodies (21).

A more likely possibility is that alloantibodies activate a regulatory cell population in the F1 host, probably because protection can be adoptively transferred into syngeneic recipients. Spleen cells from mice previously treated with alloantibodies provide protection from GvH-associated suppression equal to that produced by injection of the recipients with antibodies. Therefore, it appears that such a regulatory effect is an active process triggered by antibodies specific for either haplotype expressed by the F1 mice. Since antibodies specific for both parents in the (B × A)F1 protected against suppression induced by parent A, the activation step need not be specific only for the haplotype of the parental cells inoculated. The function of this cell population in the GvH model seems to be to prevent suppression and perhaps to restore immune reactivity following severe GvH-associated immunosuppression.

GvH-associated immunosuppression is triggered by the injected parental spleen cells that recognize and react against the other parental haplotype in the F1 host (3, 4). Such A-anti-B immune reactivity in the (B × A)F1 would be recognized by the host as anti-self reactivity. In the process of protecting itself against this "autoimmune" response, the entire immune system would be suppressed, because GvH-associated immunosuppression appears to be nonspecific and affects cell-mediated (8, 9) as well as humoral immunity (22). To counter such drastic suppression, it could be advantageous to have a regulatory cell population capable of restoring normal immune functions. Similar regulatory networks have been reported for antibody (23) and T cell responses (24). The latter has been described in F1 rats, which could be protected against GvH by pretreatment with suboptimal doses of parental T cells. However, the regulatory system in rats is rapidly activated (within 2 d), and it is specific for the immunizing parental haplotype. In contrast, the regulatory system described in the present report is activated by antibodies, which would probably require a longer period if generated in vivo by the injected parental cells.

The present study indicates that such a regulatory cell population is activated by antibodies against host antigens. In addition, the injection of parental T cells into F1 mice can result in the production of autoantibodies, which were reported to be generated by F1 B cells (25). This phenomenon is based on the allogeneic effect, in which parental T cells can activate antibody production by F1 B cells (26). Although in the present study we have injected alloantibodies specific for F1 MHC antigens, it is possible that other antibodies against different host cell surface antigens, e.g., autoantibodies generated by F1 B cells as a result of the allogeneic effect, could reverse GvH-associated suppressed T cell immunity. Studies are in progress to test this possibility. Thus, the recovery of the suppressed immune function of F1 mice undergoing a chronic GvH reaction could be due to activation of regulatory T cells by anti-MHC antibodies produced in situ by parental B cells and/or autoantibodies produced by F1 B cells. Under more natural conditions such regulatory cells may be one component of a complex regulatory network composed of suppressor cells and
regulatory cells activated by antibodies against self-components. In this model, it would be expected that the presence of autoantibodies is paralleled by low suppressor cell activity. This, in fact, is the case in the autoimmune NZB/W mouse strains (27). Such low suppressor cell activity might be due to the activation of regulatory cells (activated by autoantibodies), which serve to counteract the effect of suppressor cells. Studies are in progress to characterize the regulatory cells and to analyze the signals involved in activation of these regulatory events. A more thorough understanding of this system may be useful for treatments of GvH diseases in humans.

Summary

Injection of parental spleen cells into unirradiated F1 hybrid mice results in suppression of the potential to generate cytotoxic T lymphocyte (CTL) responses in vitro. In an attempt to protect the F1 mice from immunosuppression, the recipients were injected with antibodies specific for major histocompatibility complex (MHC)-encoded antigens of the F1 strain 24 h before inoculation of the parental spleen cells. 8–14 d later, the generation of CTL responses in vitro against H-2 alloantigens was tested. Alloantiserum directed against either parental haplotype of the F1 strain markedly diminished the suppression of CTL activity. Furthermore, monoclonal antibodies recognizing H-2 or Ia antigens protected the F1 mice from parental spleen cell-induced suppression. Although this study has been limited to reagents that recognize host H-2 determinants, these findings do not necessarily imply that protection against graft vs. host (GvH) can be achieved only with anti-MHC antibodies. However, protection was observed only by antibodies reactive with F1 antigens, and small amounts of the alloantibodies were sufficient to diminish CTL suppression. Adoptive transfer of spleen cells from syngeneic F1 mice treated with anti-H-2 alloantiserum 24 h previously provided protection equal to that of injection of the recipients with alloantibodies. The cells necessary for this effect were shown to be T cells and to be radiosensitive to 2000 rad. This cell population is induced by antisera against F1 cell surface antigens and effectively counteracts GvH-associated immunosuppression.

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