GRAFT-VERSUS-HOST-RELATED IMMUNOSUPPRESSION IS INDUCED IN MIXED CHIMERAS BY ALLORESPONSES AGAINST EITHER HOST OR DONOR LYMPHOHEMATOPOIETIC CELLS

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Graft-versus-host disease (GVHD) in man and animals is associated with immune dysfunction, which results in significant morbidity and mortality (1). However, the precise role of graft-versus-host (GVH) reactivity in producing immune dysfunction is difficult to evaluate, because ablation of host immunity is necessary before GVHD can be induced. Examination of the effects of GVH reactions without reciprocal reactions of host aganist donor have involved the use of recipients that are not immunocompetent, such as neonatal mice (2), and adult animals in which the immune system has been artificially ablated by lethal or sublethal irradiation (3-6). In addition, GVH reactions have been induced in recipients that are initially immunocompetent but specifically tolerant of donor inocula. For example, GVHD has been induced by injection of donor lymphocytes into adult rodents neonatally tolerized to donor antigen (7, 8), and the effects of GVH inocula on immune function have been extensively studied in immunocompetent F_1 animals inoculated with parental lymphocytes (9-13). Loss of immune responsiveness after inoculation of F_1 recipients with parental lymphocytes has been variably attributed to suppressor cells (9-11), destruction of recipient lymphocytes (11, 14, 15), and thymic injury (9, 12, 13). However, the contribution of each of these mechanisms has not been well defined. We have now studied the effects of GVH reactions on immune responsiveness in animals with otherwise intact immune systems, in which tolerance of recipients to the donor inoculum is not due to a genetic mechanism, but has instead been induced by lethal irradiation followed by reconstitution with T cell–depleted (TCD) bone marrow (BM). Comparison of the effects of GVH inocula in fully allogeneic vs. mixed allogeneic chimeras as well as examination of the effects of host vs. graft (HVG) inocula have allowed examination of the role of recipient lymphohematopoietic cells, in distinction from the remainder of the animal's tissues, in inducing the immune abnormalities associated with GVH reactions in vivo.

Materials and Methods

Radiation bone marrow chimeras were prepared as previously described (16, 17). Briefly, recipient C57BL/10SnJ (B10) or B10.D2nSn (B10.D2) mice, 12-14 wk old, received lethal whole body irradiation and were reconstituted with allogeneic (B10 or B10.D2) with or without syngeneic (recipient strain) TCD bone marrow. Appropriate levels of chimerism were ascer-

tained in all chimeric responders by H-2 phenotyping of PBL by fluorescence microfluorim-etry performed 6–10 wk after bone marrow transplantation (BMT) (18). Animals receiving 5 × 10⁶ TCD syngeneic plus 1.5 × 10⁷ TCD allogeneic BM cells reconstituted as stable mixed chimeras, demonstrating 25–75% allogeneic lymphoid chimerism, and are referred to here as "mixed" chimeras. Animals reconstituted with 1.5 × 10⁷ TCD allogeneic cells alone are designated "allogeneic" chimeras and demonstrated 94–99% allogeneic chimerism. Previous studies have established the presence of specific tolerance to donor and host antigen and per-manent, stable chimerism in such animals (16, 17). GVH inocula consisting of 2–3 × 10⁷ splenocytes from normal donor-strain mice were administered via the lateral tail vein 10–22 wk after BMT. These recipients, as well as uninjected control chimeras, were killed 12 d after inoculation, and cell-mediated lympholysis (CML) responses of their splenocytes were as-sessed as previously described (19).

Results and Discussion

GVH inocula consisting of 2–3 × 10⁷ splenocytes from normal donor-strain mice were administered to long-term stable chimeras. Fig. 1 (*upper panels*) shows typical CML responses of a long-term B10 + B10.D2 → B10 mixed allogeneic chimera 12 d after inoculation with B10.D2 splenocytes. The uninjected animal was unrespon-sive to donor (B10.D2) and host (B10) antigen, but responded normally to third-
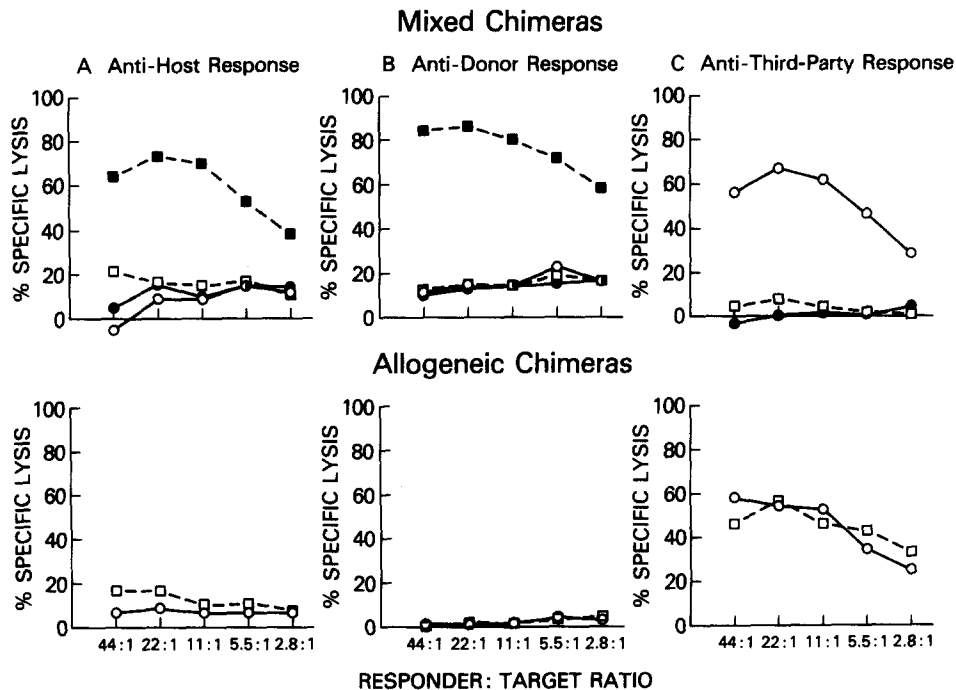

FIGURE 1. Cell-mediated lympholysis (CML) responses of chimeras against (*A*) host (B10), (*B*) donor (B10.D2), and (*C*) third-party (B10.BR) alloantigen 12 d after inoculation with normal donor-strain (B10.D2) spleen cells (□) or in unmanipulated control chimeras (O). Positive con-trol responses (■) of normal (*A*) B10.D2 or (*B*) B10 spleen cells as well as negative control re-sponses (●) of normal (*A*) B10, (*B*) B10.D2, or (*C*) B10.BR spleen cells are also shown. Irradiated stimulator cells and target blasts (stimulated with Con A for 2 d in culture) were obtained from normal B10 mice in *A*, from B10.D2 mice in *B*, and from B10.BR mice in *C*. Upper panels show responses of mixed chimeras (B10 + B10.D2 → B10), and lower panels show responses of an al-logeneic chimeras (B10.D2 → B10) given an identical B10.D2 spleen cell inoculum at the same time, as well as those of an unmanipulated allogeneic chimera.

party (B10.BR) alloantigen. The injected chimera, in contrast, was severely hyporesponsive towards third-party alloantigen. Similar anti–third-party CML hyporesponsiveness has been observed in 17 of 17 mixed chimeras tested from 10 to 28 d after administration of GVH inocula. Anti–third-party alloresponsiveness was lost between 5 and 10 d after inoculation, and recovered in most animals by 7–12 wk (data not shown). Similar to uninjected control chimeras, the injected mixed chimera shown in Fig. 1 was unresponsive to donor antigen. It did, however, demonstrate slight CML reactivity towards host antigen, which was a common but variable finding among injected mixed chimeras.

Alloresponses were studied in B10.D2 → B10 (allogeneic) chimeras after administration of similar GVH inocula, and compared with those of allogeneic chimeras not receiving GVH inocula. Unlike mixed chimeras given identical inocula in the same experiments, allogeneic chimeras retained CML responsiveness to third-party alloantigen (Fig. 1 C, lower panel). Such responsiveness was maintained at all time points tested (5, 10, 12, 13, 21, and 28 d after GVH inoculum). Injected allogeneic chimeras also remained unresponsive to donor antigen, and some demonstrated slight CML reactivity towards host antigen.

Therefore, these studies demonstrated that mixed chimeras, when inoculated with nontolerant donor-strain cells, lost the ability to generate CTL in response to third-party alloantigen. These results are similar to those described in F_1 recipients, in which administration of parental cells recognizing F_1 class I plus class II alloantigens results in loss of CML responses to third-party alloantigen (10, 11, 14, 20). In contrast, when donor-strain lymphocytes were injected into allogeneic chimeras, CML responses of recipient chimeras to third-party alloantigen were not significantly affected. These findings suggested that the development of GVH-related immunodeficiency might depend on the presence of substantial numbers of lymphohematopoietic cells expressing the recipient phenotype, and that the recognition of such antigen on other somatic cells of the host was not sufficient to induce immunodeficiency. We reasoned that if this were true, then the presence of a one-way response to donor-type lymphohematopoietic cells might also be sufficient to lead to immune hyporesponsiveness, in this case induced by administration of nontolerant recipient-strain lymphocytes to mixed chimeras (i.e., HVG inocula). Such mixed chimeras would provide donor BM-derived lymphohematopoietic cells as a source of stimulating antigen, and should be tolerant of recipient antigen (16, 17), and therefore, should not react against injected recpient-strain lymphocytes.

To test this hypothesis, 3×10^7 recipient-strain splenocytes were injected into long-term B10 + B10.D2 → B10 and B10 + B10.D2 → B10.D2 mixed chimeras. As a control, additional mixed chimeras of both types received GVH inocula (3×10^7 donor-strain, B10 or B10.D2 splenocytes) in the same experiment. Representative anti–third-party alloresponses from such animals 12 d after inoculation are shown in Fig. 2. Injection of either B10 or B10.D2 splenocytes into mixed chimeras of either B10 or B10.D2 recipient strains resulted in marked hyporesponsiveness to third-party alloantigen, irrespective of whether the splenocytes represented a GVH or an HVG inoculum. Uninjected control animals, in contrast, remained responsive to third-party alloantigen. Thus, the presence of a one-way alloresponse to either donor or host antigen on lymphohematopoietic cells in vivo was sufficient to induce hyporesponsiveness to third-party alloantigen, indicating that induction of unresponsiveness does not specifically require a GVH reaction. It therefore seems likely that induction of GVH-related immunodeficiency described in parent into F_1 systems (9–12, 15, 20)
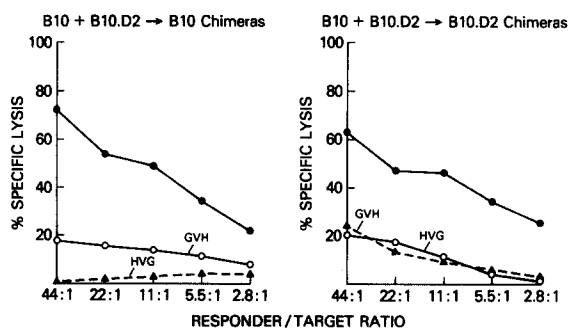
FIGURE 2. Effects of GVH and HVG inocula on anti–third-party CML responses in mixed allogeneic chimeras. Splenocytes were removed from mixed B10 + B10.D2 → B10 (*left panel*) and B10 + B10.D2 → B10.D2 (*right panel*) chimeras 13 d after inoculation with B10 (▲) or B10.D2 (○) lymphocytes, or after no injection (●). Curves represent percent specific lysis at various responder/target ratios of chimeric splenocytes on B10.BR Con A blasts after incubation for 5 d with irradiated B10.BR stimulator cells (see legend to Fig. 1). Similar results were obtained in three independent experiments.

might not require a generalized GVH reaction, but instead might reflect a one-way immune response in vivo to host lymphohematopoietic components.

These results are incompatible with one possible explanation for the immune dysfunction observed in F_1 animals inoculated with parental lymphocytes, namely the destruction of recipient T cells by the donor inoculum (11, 14) with selective overgrowth of host-reactive donor-derived T cells (15). Mixed chimeras contain T cells derived from both donor and recipient strain BM, and preliminary data indicate that both types of T cells are capable of mediating CTL responses (unpublished data). Therefore, those donor- or recipient- BM–derived T cells that are capable of mediating immune responses against third-party alloantigen but that are not potential targets of GVH- or HVG-reactive cells, respectively, should persist after GVH or HVG inoculation. The apparent inability of these cells to respond to third-party alloantigen suggests that they may in some way be inactivated as a result of the GVH reaction. Preliminary studies suggest this may be due to the induction of a G10-adherent suppressor cell population (Sykes et al., unpublished data).

Complete repopulation with allogeneic cells occurred in most mixed chimeras by 30–60 d after administration of GVH inocula. Although such repopulation is evidence for the occurrence of antihost aggressive activity in vivo, these animals survived long-term with no clinical evidence of GVHD. The numbers of donor-strain lymphocytes administered in GVH inocula in these experiments greatly exceeded those required to produce lethal GVHD in fresh irradiated animals (21). It is therefore possible that suppressive activity producing the immune hyporesponsiveness described here may also protect recipients from GVHD. However, tolerance to donor alloantigen is rapidly broken in recipients of similar HVG inocula to those used here (22), indicating that the immunosuppression resulting from such inocula is not effective in preventing the alloreactivity associated with graft rejection. Alternative explanations for the absence of GVHD in mixed chimeric recipients of GVH inocula, therefore, also deserve consideration. Induction of clinical GVHD may require factors in addition to one-way immune responses against host lymphohematopoietic cells in vivo, such as the acute tissue injury and infections that may follow lethal irradiation (9). It is also possible that a form of alloresistance against donor lymphoid cells exists in mixed chimeras similar to that which protects F_1 mice from the induction of GVH-related effects by parental lymphocytes of certain strains (20).

In summary, the studies presented here suggest a requirement for lymphohematopoietic cells bearing host antigen in the induction of GVH-related immunosup-

pression in radiation chimeras. A similar defect in immune responses was found in mixed chimeras given inocula that attack donor and not recipient antigens. These findings are most consistent with the interpretation that the immunodeficiency associated with GVH reactions is due to the development of suppressive activity as a consequence of a strong one-way alloresponse directed against lymphohematopoietic cells in vivo, whether this be an HVG or a GVH response. The presence of such immune suppression in mixed chimeras did not lead to death from infection, and did not appear to prevent the rapid loss of tolerance to donor antigen in animals given HVG inocula (22). The apparent discrepancy between the tolerance-breaking studies (22) and those reported here might either be due to differences in the time courses of the in vitro and in vivo phenomena, or might indicate that in vitro unresponsiveness does not accurately reflect the in vivo immune status of such recipients. Further studies will be required to elucidate the in vivo consequences, if any, of the immune unresponsiveness observed in vitro after administration of HVG and GVH inocula. One potential consequence to be addressed is the possibility that in vivo reactivity against injected lymphohematopoietic cells is responsible for the nonspecific immunosuppression that appears to result after blood transfusion in prospective organ transplant recipients (23).

Summary

Graft-vs.-host (GVH)-related immunosuppression has previously been demonstrated in F_1 rodent recipients of parental lymphoid cells, and has been thought to result from an immunologic attack of the donor against the host. Since all cells of such F_1 recipients could potentially bear target class I MHC alloantigens, it has not previously been possible to determine precisely the target tissues responsible for development of GVH-related effects. In the present studies we have used mixed allogeneic chimeras as recipients of host or donor-strain lymphocyte inocula, and have made the surprising observation that "GVH-induced" immune unresponsiveness does not require GVH reactivity, per se, but develops in the presence of a one-way alloresponse against lymphohematopoietic cells in either the GVH or the host-versus-graft direction.

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