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Target Antigens Determine Graft-versus-Host Disease Phenotype

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Chronic graft-vs-host disease (cGVHD) is an increasingly frequent complication of allogeneic stem cell transplantation. Phenotypically, cGVHD differs from patient to patient; in particular, a subset of patients develops extensive cutaneous fibrosis. Similarly, graft-vs-host disease (GVHD) is distinct in inbred murine donor:recipient pairings, indicating a genetic component to disease phenotype. The B10.D2 → BALB/c (H-2^{d}) strain pairing uniquely recapitulates key pathologic features of fibrotic human cutaneous cGVHD. To distinguish whether this genetic component is due to differences in genes that modulate immune responses or to the specific Ags targeted, we asked whether skin-dominant cGVHD also develops in the B10 → BALB.B (H-2^{b}) and B10.BR → BALB.K (H-2^{k}) MHC-congenic pairings. Because each MHC haplotype presents different peptides and selects different T cell repertoires, GVH in each donor:recipient pair undoubtedly targets different Ags. We found that, in contrast to BALB/c recipients, BALB.B mice never manifested skin disease while BALB.K mice developed a modified form of skin disease. Instead, BALB.B and BALB.K recipients developed systemic GVHD which was absent in BALB/c mice. Moreover, in (B10 × B10.D2)F_{1} → (BALB.B × BALB/c)F_{1} H-2^{k/b} transplants, recipients developed both cutaneous and systemic disease. Thus, the selection of immunodominant Ags determines the target and character of GVHD, providing insight into the genetic basis for different forms of GVHD. The Journal of Immunology, 2004, 173: 5467–5475.

Allogeneic stem cell transplantation is a potentially curative therapy for hematologic malignancies, inherited disorders of stem cells including sickle cell anemia and acquired nonmalignant diseases such as aplastic anemia (1–11). However, the application of allogeneic stem cell transplantation has been limited by the morbidity and mortality of graft-vs-host disease (GVHD)^{9} (12). Broadly, GVHD is divided into acute (aGVHD) and chronic (cGVHD) forms. For epidemiologic purposes, aGVHD begins before day 100 after transplant and cGVHD begins after day 100 (13). aGVHD and cGVHD also have distinct clinical and pathologic features (14, 15). aGVHD presents more abruptly than cGVHD and is characterized by diarrhea and an inflammatory infiltration of skin, bowel, and liver. In contrast, cGVHD has a more slowly progressive course and a wider range of clinical and pathologic features often characterized by fibrosis and scleroderma-like changes. Salivary and lacrimal gland involvement are common and more protean manifestations such as bronchiolitis and eosinophilic fasciitis are well documented (15–22).

Although numerous murine GVHD models exist, those that are MHC-identical and involve transplantation across only minor histocompatibility Ag (miHAs) differences most accurately mirror clinical practice. Seminal studies by Korngold and Sprent (23–27) and Hamilton (28) demonstrated that different MHC-identical donor:recipient strain combinations developed distinct syndromes of GVHD, most of which were acute with a variable dependence on CD4^{+} and CD8^{+} T cells. Thus, even though environmental factors can affect severity and penetrance, there is clear evidence that genetics plays a key role in the development of GVHD. We therefore were interested in examining the genetic basis of GVHD phenotypes.

We have recently revisited one of these strain combinations, the B10.D2 → BALB/c (H-2^{d}) minor Ag mismatch model of GVHD (29, 30). This strain pairing is unique because BALB/c recipients of B10.D2 bone marrow and spleen cells develop a chronic-type, CD4^{+} T cell-dependent form of GVHD characterized by a relatively late time of onset, skin fibrosis, ulceration, and alopecia with increased collagen deposition at least in part due to TGF-β (27, 30–33). Pulmonary fibrosis, biliary cirrhosis, and destruction of lacrimal as well as salivary glands also develops (34). Because of its clinical and pathological similarities to human cGVHD, we and others have used this as model to study cGVHD.

Two nonexclusive hypotheses could explain the different GVHD syndromes seen in both human and murine GVHD as epitomized by the unique GVHD phenotype seen in B10.D2 → BALB/c transplants. One possibility is that a particular GVHD phenotype results from genetic differences in “background” genes that determine the inherent nature of immune responses. There are several mechanisms by which background genes could influence immune responses and GVHD phenotype. For instance, levels of cytokines that determine T cell polarization could modulate GVHD. Indeed, some believe that cGVHD is a Th2-type disease, whereas aGVHD results from Th1 T cells (35). There is also evidence that different incidences of GVHD are associated with polymorphisms in the regulatory regions of the genes for TNF-α, TNFR, IL-10, IFN-γ,
IL-6, and TGF-β (36–40). A second possibility is that the identity and tissue distribution of immunodominant target Ag(s) dictates GVHD phenotype. For example, in the B10.D2 \(\rightarrow\) BALB/c model, skin GVHD could result from cutaneous expression of immunodominant Ags whereas the acute GVHD seen in other strain combinations could be the result of expression of immunodominant Ags in other tissues such as biliary or intestinal epithelia.

One way to distinguish between these hypotheses would be to compare GVHD in models in which the genes that govern immune responses are held constant but the target Ags vary. To make this distinction, we compared GVHD in three MHC-identical strain pairings that have identical background genes and differ only at their MHC loci: B10.D2 \(\rightarrow\) BALB/c (H-2\(^b\)), B10 \(\rightarrow\) BALB.B (H-2\(^k\)), and B10.BR \(\rightarrow\) BALB.K (H-2\(^d\)). Changing MHC has two important effects. First, each MHC will present different sets of peptides based on differences in the residues that govern peptide binding (41). In this way, changing MHC leads to different Ags being presented. Second, different MHC (with their different peptides) will select a different TCR repertoire. Therefore, the net effect of changing MHC is that alloimmune T cells in each MHC-congenic strain pairing will focus on different Ags. Thus, by comparing GVHD in these strain pairings, we are testing the impact of changing target Ags while holding constant genes that might regulate T cell responses.

Strikingly, we found that each congenic pair manifested a distinct form of GVHD with unique clinical and pathologic features and different abilities of CD4\(^+\) and CD8\(^+\) T cells to induce disease. In particular, chronic, fibrotic skin-targeted GVHD required H-2\(^b\) for maximal expression and was completely absent in H-2\(^d\) mice. Moreover, (B10 \(\times\) B10.D2)F\(_1\) \(\rightarrow\) (BALB.B \(\times\) BALB/c)F\(_1\) (H-2\(^{k/b}\)) animals developed a hybrid form GVHD with features similar to that found in both B10.D2 \(\rightarrow\) BALB/c (H-2\(^d\)) and B10 \(\rightarrow\) BALB.B (H-2\(^b\)). Taken together, these data argue that genes in the MHC locus, most likely through MHC-based selection of immunodominant Ags, can dominantly determine the character of GVHD.

**Materials and Methods**

**Mice**

BALB/c (H-2\(^b\)) were purchased from the National Cancer Institute Laboratories (Frederick, MD) and Harlan Breeders (Indianapolis, IN). BALB.B (H-2\(^d\)), BALB.K (H-2\(^k\)), C57BL/10 (H-2\(^s\)), and B10.BR (H-2\(^b\)) were purchased from Harlan Breeders. B10.D2 (H-2\(^a\)) were purchased from The Jackson Laboratory (Bar Harbor, ME) and Harlan Breeders. (BALB/c \(\times\) BALB.B) and (B10 \(\times\) B10.D2) were generated by breeding the indicated parent strains to generate F\(_1\) animals. Eight- to 12-wk-old male mice were used in experiments. Mice were housed in microisolator cages and fed nonautoclaved food and acidified water containing Sulfatrim for 2 wk posttransplant.

**Bone marrow (BM) transplantation**

Recipient BALB/c, BALB.B, BALB.K, and (BALB/c \(\times\) BALB.B) mice received 775 cGy from a cesium irradiator and were reconstituted with 8 \(\times\) 10\(^7\) T cell-depleted BM with spleen cells or purified T cell subsets (as described below) from B10.D2, B10, B10.BR, or (B10.D2 \(\times\) B10 donors), respectively.

**BM T cell depletion**

BM cells were isolated and Thy1.2-positive T cells were depleted using an AutoMACS (Miltenyi Biotec, Bergisch Gladbach, Germany) as previously described (30). Remaining Thy1.2-positive cells were routinely <0.5% of BM cells. Cells were resuspended in injection buffer (1X PBS, 10 mM HEPES, 2.5% acid citrate dextrose antimicrobial, and 0.5% penicillin-streptomycin) before transplant.

**T cell purifications**

Spleens from donor mice were crushed through 70-μm screens in MACS buffer (1X PBS, 5 mM EDTA, and 3% calf serum). After RBC lysis, spleen cells were resuspended in injection buffer before transplant or underwent further cell purifications (see below). Before injection, unfractionated spleen cells were assayed by FACS using anti-CD8-PE (BD Pharmingen, San Diego, CA) and anti-CD4-FITC (GK 1.5, laboratory conjugated) to monitor the ratios of CD4\(^+\) and CD8\(^+\) T cells in the various strains. These were similar in all experiments, with CD4\(^+\) and CD8\(^+\) cells representing 11–14% and 6–8%, respectively, of total spleen cells.

**FIGURE 1.** Congenic B10.D2 \(\rightarrow\) BALB/c, B10 \(\rightarrow\) BALB.B, and B10.BR \(\rightarrow\) BALB.K develop different forms of cutaneous GVHD. a. On day 0, groups of 14 BALB/c (H-2\(^d\), thick solid line) and 15 BALB.B (H-2\(^b\), dashed line) recipients were lethally irradiated and reconstituted with MHC-identical 8 \(\times\) 10\(^7\) T cell-depleted BM and 10\(^7\) spleen cells from B10.D2 (H-2\(^a\)) or B10 (H-2\(^b\)) donors, respectively. Control groups of five BALB/c and BALB.B mice were reconstituted with BM alone and are represented together (thin solid line). All mice were monitored for clinical cutaneous GVHD and are represented as the percentage of mice free from disease. *, p < 0.001 BALB/c vs BALB.B. b. As in a, groups of 12 BALB/c (H-2\(^k\), thick solid line) and 12 BALB.K (H-2\(^k\), dashed line) recipients were lethally irradiated and reconstituted with 8 \(\times\) 10\(^7\) BM and 10\(^7\) spleen cells from B10.D2 (H-2\(^a\)) or B10.BR (H-2\(^b\)) donors, respectively. Control groups of five BALB/c and BALB.K mice were reconstituted with BM alone (thin solid line), *, p < 0.001 BALB/c vs BALB.B. c. Data represent pathologic GVHD scores for back skin obtained from BALB/c, BALB.B, and BALB.K recipients. Each symbol represents the score from an individual animal. Data from recipients of total spleen cells from a and b have been combined with data from recipients of total spleen cells from Fig. 2 to more completely represent the range of pathology seen. Mean represented by horizontal bar. Cutaneous pathology scores from BALB.B differ significantly from BALB/c and BALB.K (p < 0.0001). BMT, BM transplantation.
To purify CD4<sup>+</sup> and CD8<sup>+</sup> T cells, donor spleen cells were incubated with microbeads directly conjugated to either anti-CD4 or anti-CD8 (Miltenyi Biotec) for 20 min on ice followed by positive selection using an AutoMACS. Positively selected cells were routinely 90% CD4<sup>+</sup> or CD8<sup>+</sup> cells. To deplete CD8<sup>+</sup> T cells, donor spleen cells were incubated with biotinylated anti-CD8 (TIB105) followed by streptavidin-conjugated microbeads (streptavidin beads; Miltenyi Biotec) and negative selection using an AutoMACS. Contaminating CD8<sup>+</sup> T cells were routinely <0.2% of spleen cells.

**GVHD clinical scoring system**

Beginning on day 10 after transplantation, animals were scored every 3–4 days for systemic and cutaneous manifestations of GVHD. Animals were monitored for the development of alopecia, erosions, and ulcers on their hair-bearing skin. Animals were scored as having cutaneous GVHD if they developed alopecia >1.0 cm<sup>2</sup>, erosions, or ulcerations in hair-bearing areas. Non-hair-bearing skin (ears and tails) was also examined for evidence of erosions or scaling. Conjunctiva were also examined for evidence of GVHD (edema, inflammation, and crusting). For systemic GVHD, animals were monitored for hunched posture and diarrhea which was detected by the presence of residual perianal fecal material. Animals found to have severe hunched posture or diarrhea on two consecutive observations were scored as having systemic GVHD. Once a mouse was scored as having GVHD, it was always considered affected even if the symptoms of GVHD subsided or if the mouse died or was euthanized for humane reasons. Similarly, although infrequent, animals that died without clinical evidence of GVHD were always considered unaffected.

**GVHD pathologic scoring**

Shaved skin from the interscapular region (~2 cm<sup>2</sup>), liver, and descending hair-bearing skin. Animals were scored as having cutaneous GVHD if they developed alopecia>1.0 cm<sup>2</sup>, erosions, or ulcerations at hair-bearing areas. Non-hair-bearing skin (ears and tails) was also examined for evidence of erosions or scaling. Conjunctiva were also examined for evidence of GVHD (edema, inflammation, and crusting). For systemic GVHD, animals were monitored for hunched posture and diarrhea which was detected by the presence of residual perianal fecal material. Animals found to have severe hunched posture or diarrhea on two consecutive observations were scored as having systemic GVHD. Once a mouse was scored as having GVHD, it was always considered affected even if the symptoms of GVHD subsided or if the mouse died or was euthanized for humane reasons. Similarly, although infrequent, animals that died without clinical evidence of GVHD were always considered unaffected.

**Results**

**Congenic B10.D2 → BALB/c, B10 → BALB.B, and B10.BR → BALB.K develop three unique forms of cutaneous GVHD**

We first compared GVHD in the B10 → BALB.B (H-2<sup>b</sup>) and B10.D2 → BALB/c (H-2<sup>d</sup>) strain pairings. Using identical transplant protocols, recipients were irradiated and reconstituted with T cell-depleted BM with or without 10<sup>7</sup> unfractionated spleen cells and then followed for the development of GVHD. As expected (30), almost 80% of BALB/c spleen cell recipients developed erosions and areas of alopecia (Fig. 1a). Tail scaling and ear erosions were also noted in 86% of BALB/c mice. Strikingly, BALB.B mice failed to manifest any clinical evidence of cutaneous GVHD (Fig. 1a, p < 0.001 BALB.B vs BALB/c). Control mice that received BM without additional splenocytes did not develop cutaneous GVHD. Histologic examination of skin confirmed the presence of cutaneous GVHD in BALB/c and the absence of subclinical cutaneous disease in BALB.B mice (Fig. 1c, p < 0.0001 BALB.B vs BALB/c).

We next compared GVHD in the B10.D2 → BALB/c strain pairing using animals obtained from our standard suppliers (The Jackson Laboratory and National Cancer Institute, respectively) with animals obtained from Harlan Breeders which is our standard supplier of BALB.B mice. Incidence and severity of GVHD using mice from either source were indistinguishable (data not shown). This rules out the possibility that the phenotypic differences observed above (Fig. 1) were the result of genetic drift or different husbandry conditions in the separate mouse colonies.

To extend our findings, we examined GVHD in the B10.BR → BALB.K (H-2<sup>k</sup>) strain pairing. In this experiment, all of the BALB/c mice developed cutaneous GVHD between weeks 3 and 4 posttransplantation while only 2 (17%) of 12 BALB.K mice developed cutaneous GVHD (Fig. 1b, p < 0.001). Unlike BALB/c mice, the two BALB.K mice with cutaneous disease manifested disease at a much later point (5–6 wk posttransplantation). Although only two of the BALB.K mice developed clinical GVHD as defined by alopecia, erosions, or ulcers in hair-bearing areas, 9 (75%) of 12 developed ear erosions and tail scaling similar to those seen in BALB/c mice. Histologic examination of hair-bearing skin revealed evidence of GVHD in BALB.K recipients even in the absence of alopecia, erosions, or ulcers (Fig. 1c). Nonetheless, although BALB.K mice had histologic evidence of skin GVHD, in each of the five scored categories (Table I), scores were lower than in BALB/c spleen cell recipients (p < 0.05). Aside from differences in clinical manifestations, BALB.K mice clearly had a qualitatively distinct syndrome from BALB/c mice. This was evident in destruction of hair follicles which occurred frequently in BALB/c and rarely in BALB.K mice (Table I, p < 0.0001). This difference likely explains why GVHD of hair-bearing areas was not clinically evident in BALB.K animals. Thus, even though BALB/c, BALB.B, and BALB.K are congenic strains differing by guest on April 20, 2017 http://www.jimmunol.org/ Downloaded from
only at the MHC locus, each develops a distinct form of cutaneous GVHD.

**MHC genes also control the ability of CD4⁺ and CD8⁺ T cells to generate cutaneous GVHD**

Since the three congenic strains differ at both MHC class I and MHC class II, the unique GVHD phenotypes we observed could have been due to differences in the specificities of CD4⁺ T cells, CD8⁺ T cells, or both. To more precisely examine the role of target Ag, we therefore tested the ability of purified CD4⁺ and CD8⁺ T cells to induce GVHD in each strain pairing. As we and others have reported (30), BALB/c recipients of B10.D2 CD4⁺ T cells generated disease that was indistinguishable from disease induced by unfractionated B10.D2 spleen cells (Fig. 2a), whereas CD8⁺ T cells alone did not cause clinical GVHD. Histopathologic analysis confirmed these clinical results (Fig. 2d), BALB.B recipients of either B10 CD4⁺ or CD8⁺ T cells did not have clinical or pathologic cutaneous GVHD, which was also expected, since unfractionated spleen cells did not affect skin (Fig. 2, b and d). BALB.K recipients given either CD4⁺ or CD8⁺ T cells all developed similar cutaneous GVHD to that seen in the previous experiment with unfractionated spleen cells (Fig. 2c). Histologically, BALB.K spleen, CD4⁺, and CD8⁺ T cell recipients showed evidence of cutaneous GVHD (Fig. 2d) with sparing of the hair follicles (mean follicular path score of 0, data not shown) which was consistent with the results from unfractionated spleen cells. Thus, CD4⁺ T cells are sufficient to generate cutaneous GVHD in BALB/c and BALB.K mice and CD8⁺ T cells are sufficient to cause cutaneous disease only in BALB.K mice.

**B10 → BALB.B and B10.BR → BALB.K mice develop systemic GVHD**

Our experiments were originally conceived to distinguish mechanisms of cutaneous GVHD in the three MHC-congenic strain pairings. However, as we carefully analyzed other parameters of GVHD in the above experiments, we noted other differences in GVHD manifestations that depended on MHC haplotype. In our initial set of B10 → BALB/c and B10 → BALB.B experiments using unfractionated spleen cells, none of the BALB/c mice demonstrated evidence of systemic GVHD (Fig. 3a). Strikingly, however, BALB.B recipients of B10 spleen cells all rapidly developed diarrhea and hunched posture (Fig. 3a, p < 0.001 BALB.B vs BALB/c). BALB/c recipients of purified CD4⁺ or CD8⁺ T cells did not develop systemic GVHD, which is consistent with the results using unfractionated spleen cells (Fig. 3b). In BALB.B recipients, however, B10 CD4⁺ T cells induced systemic disease similar to that produced by unfractionated splenocytes, whereas CD8⁺ cells induced a more delayed systemic GVHD with reduced incidence (Fig. 3c, p = 0.005 for CD4⁺ vs CD8⁺).

As with the BALB.B mice, systemic disease was clearly evident in 10 of 12 BALB.K unfractionated spleen cell recipients (Fig. 3d). When BALB.K recipients were transplanted with either purified CD4⁺ or CD8⁺ T cells, they developed systemic GVHD that was similar to that of BALB.K recipients given unfractionated spleen cells (Fig. 3e). In the experiment using unfractionated spleen cells (Fig. 3f), unlike earlier experiments (Fig. 3, a and b) some (5 of 12) BALB/c recipients developed systemic disease. However, it was at a significantly lower incidence (p = 0.015 BALB/c vs BALB.K). Indeed, considering all of the experiments we performed, there was a markedly lower incidence of systemic disease in BALB/c compared with either BALB.B or BALB.K mice, as summarized in Table II. Thus, as was the case for cutaneous GVHD, we observed distinct clinical syndromes of systemic GVHD in the three strain pairings.

**FIGURE 2.** Congenic pairings differ in the ability of T cell subsets to generate cutaneous GVHD. a, Groups of 10–15 BALB/c mice were lethally irradiated and reconstituted with 8 × 10⁶ T cell-depleted BM cells along with 10⁶ unfractionated spleen cells (thick solid line), 5 × 10⁶ CD4⁺ T cells (long dashed line), or 5 × 10⁶ CD8⁺ T cell (short dashed line) from B10.D2 donors. A group of five control animals received BM cells alone (thin solid line). All mice were monitored for clinical evidence of cutaneous GVHD and are represented as the percentage of mice free of disease. Groups of 12 BALB.B (b) and 6 BALB.K (c) mice were lethally irradiated and reconstituted with 8 × 10⁶ T-depleted BM cells along with 10⁶ unfractionated spleen cells (thick solid line), 1.4 × 10⁶ CD4⁺ T cells (long dashed line), or 6 × 10⁶ CD8⁺ T cell (thin solid line) from B10 (BALB.B) or B10.BR (BALB.K) donors. A group of four control animals received BM alone (thin dashed line). d, Data represent pathologic GVHD scores for back skin obtained from recipients in a–c. Each symbol represents the score from an individual animal. BMT, BM transplantation.
We analyzed pathology from bowel and liver to better understand the clinical systemic GVHD syndrome. Histologic examination of BALB.K recipients of spleen cells clearly showed evidence of more severe colonic GVHD (Fig. 3f, p = 0.0196 vs BALB/c, p = 0.003 vs BALB.B). Although we observed a much higher incidence of diarrhea and hunched posture in B10 → BALB.B compared with B10.D2 → BALB/C spleen cell recipients, we saw comparable bowel and liver pathology in mice sacrificed 52 days (Fig. 3, f and g) and 17 days (data not shown) after transplant. Although we were hoping to find a histologic correlate of GVHD-induced diarrhea, there are evidently mechanisms of diarrhea that do not involve histologic change. We most likely observed a secretory diarrhea that is known to be a clinical feature of GVHD (42).

The previous experiments were not clinically scored with specific reference to experimental groups but neither were the cage identification obscured to the observer. Although we were confident in our clinical scoring of systemic GVHD, we wanted to exclude the possibility that the two distinct phenotypes of systemic GVHD were skewed by observer bias. We therefore performed another set of simultaneous B10.D2 → BALB/c and B10 → BALB.B transplants with GVHD induced by CD4⁺ T cells in which mice were scored by two independent observers blinded to the experimental groups. As seen in previous experiments, almost all (16 of 18) BALB/c whereas none of the BALB.B mice developed cutaneous GVHD (Fig. 4a, p < 0.001 BALB/c vs BALB.B). In contrast, nearly all (13–14 of 14) BALB.B and 1/18 BALB/c mice displayed hunched posture and diarrhea (Fig. 4h, p < 0.001, BALB/c vs BALB.B). The data from each independent, blinded observer are both displayed and are virtually identical. This not only confirms the clinical differences in GVHD between the B10.D2 → BALB/c and B10 → BALB.B models, but validates the unblinded observations in the other experiments.

**Summary of clinical results**

The combined clinical results from every experiment in which recipients received either unfractionated spleen cells or purified CD4⁺ T cells are summarized in Table II. In the B10.D2 → BALB/c group, GVHD manifested primarily as alopecia and erosions on hair-bearing areas, tail scaling, and ear erosions, but much less frequently as diarrhea or hunched posture. In contrast, B10 → BALB.B mice never developed cutaneous disease (p < 0.001, BALB.B vs BALB/c) and developed diarrhea and hunched posture with a penetrance of 73% (p < 0.001, BALB.B vs BALB/c). B10 BR → BALB.K mice
rarely developed alopecia and erosions on hair-bearing areas \((p < 0.001 \text{ BALB.K vs BALB/c})\) but, like BALB/c mice, frequently developed tail scaling and ear erosions. Unlike BALB/c mice, BALB.K mice frequently had diarrhea and hunched posture \((p < 0.001 \text{ BALB.K vs BALB/c})\).

Thus, even though BALB/c, BALB.B, and BALB.K are congenic strains that vary only at the MHC locus, each produces a unique form of GVHD. Therefore genes in the MHC locus can determine the phenotype of GVHD.

**H-2\(^d\), and H-2\(^b\)-restricted epitopes are codominant**

To determine whether genes within the MHC locus can dominantly elicit a particular GVHD phenotype, we compared GVHD in B10.D2→BALB/c, B10→BALB.B, and \((B10.D2 \times B10)\text{F}_1 \rightarrow (BALB/c \times BALB.B)\text{F}_1\) transplants in which GVHD was induced by unfractionated spleen cells (Fig. 5a) or CD\(^4^+\) T cells (CD8\(^-\)depleted spleen cells; Fig. 5b). As expected from previous experiments, BALB/c animals developed primarily cutaneous GVHD (Fig. 5, a and b, left panels) and BALB.B mice developed systemic GVHD (Fig. 5, a and b, right panels). In both, F\(_1\) animals clearly developed lesions in hair-bearing areas and tail/ear erosions characteristic of GVHD in BALB/c recipients (Fig. 5, a and b, left panels; see also Table II), although there was overall more disease in the first experiment. These clinical characteristics were confirmed histologically (Fig. 5c). F\(_1\) recipients also developed systemic disease (Fig. 5, a and b, right panels; see also Table II) which was more severe than in BALB/c \((p < 0.001)\) mice and less severe than in BALB.B mice \((p < 0.001)\). Because the F\(_1\) mice develop skin GVHD similar to the BALB/c phenotype and systemic GVHD similar the BALB.B phenotype, we conclude that MHC gene control of GVHD is codominant.

**Discussion**

Given the important genetic contribution to GVHD phenotype, we wanted to determine whether the development of GVHD results from genetic differences in “background” genes that determine the inherent nature of immune responses or from differences dictated by the identity of target miHAs. To do so, we examined GVHD in three MHC identical, multiple miHA-incompatible B10→BALB H-2-congenic strain pairings. We found three distinct forms of disease: B10.D2→BALB/c (H-2\(^d\)) mice developed primarily cutaneous, chronic-type GVHD; B10→BALB.B (H-2\(^b\)) mice failed to develop any skin GVHD and instead manifested systemic disease; and B10.BR→BALB.K (H-2\(^k\)) recipients developed an intermediate phenotype with systemic and a modified form of skin GVHD. Moreover, CD\(^4^+\) and CD\(^8^+\) T cells showed a differing ability to induce disease in the three strain pairings. Finally, we showed that \((B10.D2 \times B10)\text{F}_1 \rightarrow (BALB/c \times BALB.B)\text{F}_1\) recipients developed a hybrid form of GVHD that included both skin and systemic disease, suggesting that H-2\(^b\) and H-2\(^d\) dictate GVHD phenotype in a codominant fashion.

Different alleles of MHC present different sets of peptides to T cells (41, 43, 44). These MHC proteins (with their different peptides) will also select a different TCR repertoire. Thus, the most likely explanation for our results is that by varying MHC alleles, different sets of peptides are presented to T cells, thereby resulting in different GVHD responses. It is at first surprising that MHC changes could alter phenotype, given the large number of possible polymorphic peptide Ags. If a large number of Ags were targeted, it would be very unlikely that changing target Ags could alter which organs are affected or the pathogenicity of a particular T cell subset. The multiplicity of minor Ags would virtually guarantee that some could be recognized in each target organ regardless of MHC type. However, the three distinct GVHD phenotypes seen in each of the congenic pairs and the ability of CD\(^8^+\) T cells to cause disease in only two of the three pairings in fact argues that a small number of immunodominant Ags controls the quality of disease. Our observation of codominant disease in F\(_1\)→F\(_2\) transplants also supports this by suggesting that cutaneous disease is restricted by H-2\(^d\) and systemic disease by H-2\(^b\). Indeed, previous studies have also suggested that graft-vs-host responses are focused on a few immunodominant epitopes (45–57). Strong evidence of immunodominance in GVHD has been inferred from work demonstrating selective TCR V\(_\gamma\) usage in the B10→BALB.B strain pairing (49, 52, 54, 57). Additionally, MHC tetramers have identified a dominant and several subdominant epitopes in the same strain pairing (55). However, there were no data linking responses to a specific epitope with a distinct GVHD phenotype. In this study, we add to this previous work by demonstrating that selection of immunodominant Ag(s) can determine the quality of GVHD. To our knowledge, these results are the first to genetically link GVHD phenotype to target Ag(s).
Although the data strongly favor MHC genes themselves selecting target Ags, it is worth considering that there are a number of genes within the MHC locus other than structural MHC genes. Polymorphisms either in the coding region or regulatory elements of these genes could, in theory, be responsible for the observed phenotypic differences in GVHD. Two components of the proteosome, LMP2 and LMP7, and TAP1 and TAP2 reside in the MHC locus and can modulate the selection of peptides displayed by MHC class I (58, 59). These genes could certainly contribute to the generation and presentation of immunodominant peptides on skin or gut tissues in CD8\(^+\)/H11001 T cell-dependent GVHD. However, they would not affect Ag presentation on MHC class II molecules and thus could not explain the differences we saw in GVHD induced by CD4\(^+\)/H11001 T cells. It is also unlikely that polymorphisms in complement components in the MHC locus (C4, C2, and B) play a key role in modulating cell-mediated GVHD. The genes for TNF-\(\alpha\) and lymphotoxin A and B are also located in the MHC locus (58). Although polymorphisms in the promoter region of murine TNF which could potentially alter levels of expression have been identified, it is hard to imagine a mechanism by which levels of TNF could generate three distinct phenotypes of GVHD (60, 61). Moreover, in the F\(_1\) experiment, polymorphisms affecting cytokine levels would be expected to produce a single dominant disease phenotype rather than the codominant expression of both phenotypes that was observed.

There are several nonexclusive ways in which different target Ags could dictate GVHD phenotype. A simple explanation would be differences in Ag distribution. For example, a skin-specific immunodominant Ag in the B10.D2 \(\rightarrow\) BALB/c pairing may be highly expressed by H-2\(^d\) in BALB/c skin with lower expression in other tissues. This same Ag may not generate any polymorphic peptides in the context of H-2\(^b\). In parallel, expression of Ags in the thymus by different MHC alleles would affect both positive and negative selection, thereby generating T cell repertoires with differing ability to respond to immunodominant minor Ags in the periphery (62). Additionally, the qualitative nature of T cell
responses can also be dictated by the level of expression of an Ag on APCs and by the affinity of the TCR for these Ags. High TCR affinity and high levels of Ag expression are associated with Th1 responses whereas lower levels are more likely to result in Th2 cytokine production (63). Ag-induced alterations in T cell polarity could in turn contribute to changes in GVHD phenotype.

In this study, we have distinguished between two models for how genes can determine GVHD phenotype. The fact that MHC type and thus immunodominant target Ags control phenotype has some potential clinical applications. It has been proposed that the incidence of GVHD can be predicted based on miHA differences (64). Our results raise the possibility that in addition to incidence, the specific quality of disease could also be predicted. Prior knowledge of the tissues likely to be affected by GVHD would allow for more targeted surveillance of disease, prophylaxis, and earlier therapeutic intervention (65).

References


