Reertoire Analysis of CD8+ T Cell Responses to Minor Histocompatibility Antigens Involved in Graft-Versus-Host Disease

Thea M. Friedman, Michael Gilbert, Constance Briggs and Robert Korn gold

J Immunol 1998; 161:41-48; ;
http://www.jimmunol.org/content/161/1/41

References
This article cites 36 articles, 15 of which you can access for free at:
http://www.jimmunol.org/content/161/1/41.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Repertoire Analysis of CD8+ T Cell Responses to Minor Histocompatibility Antigens Involved in Graft-Versus-Host Disease

Thea M. Friedman, Michael Gilbert, Constance Briggs, and Robert Korngold

Graft-vs-host disease (GVHD) is a major complication of allogeneic bone marrow transplantation. Experimentally, lethal GVHD can be induced in MHC-matched strain combinations differing in expression of multiple minor histocompatibility Ags (miHA). Recently, the GVHD potential of C57BL/6By (B6) T cells in irradiated BALB.B (both H2b) and related CXB recombinant inbred strains of mice has been studied to determine the scope of the response to miHA in vivo and how it compared with CTL responses to immunodominant miHA in vitro. The GVHD response in these strain combinations appeared to be limited to a few Ags, yet there was no correlation of these miHA with that of in vitro CTL responses. To further investigate the role of CD8+ T cells in GVHD, we analyzed positively selected miHA-specific donor CD8+ thoracic duct lymphocytes (TDL) collected from irradiated BALB.B and CXBE mice, 5 to 6 days after transplantation of B6 T cells. Flow cytometric analysis of B6→BALB.B TDL did not indicate expansion of any particular TCR Vβ family, whereas Vβ10 and Vβ14 families were significantly expanded in the B6→CXBE TDL. However, PCR-based complementarity-determining region 3 size spectratyping revealed overlapping involvement of donor Vβ1, 6, 8, 9, 10, and 14 families in both BALB.B and CXBE recipients and unique utilization of the Vβ4 family in BALB.B mice, suggesting oligoclonal T cell responses to a limited number of miHA. In addition, the injection of CD8+ Vβ14+ B6 T cells into irradiated BALB.C and CXBE mice induced lethal GVHD, confirming the involvement of miHA-specific T cells within an individual Vβ family. The Journal of Immunology, 1998, 161: 41–48.

Multiple minor histocompatibility Ags (miHA) play an important role in the induction of GVHD following MHC-matched allogeneic BMT (8–11). MiHA are presented by MHC class I and class II molecules (9, 12), the former of which are recognized by CTL that can be generated in vitro by secondary stimulation of primed effector T cells (13). As an experimental system, the T cell response of C57BL/6By (B6) mice directed against miHA expressed by H2b-matched BALB.B mice has been thoroughly investigated. Despite estimated differences of more than 29 miHA loci between these two strains, it was found that the in vitro CTL response was directed to only a few immunodominant Ags (14). These BALB.B miHA were detected because of their differential expression in a panel of target cells from the CXBE, G, I, J and K recombinant inbred (RI) strain, generated from F1 crosses from an original B6 and BALB/c mating. The hierarchy of the in vitro B6 CTL reactivity indicated that CXBG and CXBK strains expressed first-order immunodominant miHA, whereas CXBE expressed second-order miHA which could stimulate a response only in the absence of the first-order Ags (14). However, GVHD studies involving the transplantation of B6 T cells and marrow into irradiated BALB.B and CXB RI strains indicated that the in vitro immunodominant miHA hierarchy did not correlate with GVHD potential (15). In contrast to strong GVHD responses observed in the BALB.B and CXB strains, GVHD was not evident in the CXBG and CXBK recipients. An interstrain GVHD response analysis with the BALB.B and CXB RI strains suggested that a minimum of two distinct MHC class I-restricted miHA (or groups of miHA) could account for induction of disease in the parental B6→BALB.B combination, designated GVH-1 and GVH-2 (16). Consistent with the observed importance of MHC class I-restricted miHA, all positive B6 GVHD responses in the BALB.B and CXB RI strains were found to involve mediation by CD8+ T cells, most of which appeared to be dependent on CD4+ T cell help for their generation; in BALB.B mice, CD4+ T cells also independently...
caused a high level of lethal GVHD (17). Furthermore, a recent phenotypic study of the B6 CD4+ TCR Vβ repertoire during the early development of GVHD in BALB.B and CXBE mice has given the first indication of involvement of a limited anti-host miHA response (18).

In the current investigation, we approached the more critical question of the nature and extent of the B6 CD8+ T cell GVHD response to miHA in the BALB.B and CXBE recipients. Positively selected miHA-specific T cell blasts were collected from the throracic duct lymphocyte (TDL) pool of irradiated BALB.B and CXBE mice, 5 days after transplantation of host-primed B6 T cells. Initial flow cytometric phenotype analysis of the CD8+ T cells in the B6→BALB.B TDL did not suggest significant expansions in any TCR Vβ family, whereas B6→CXBE TDL displayed significant expansions of the Vβ10 and Vβ14 families.

TCR Vβ repertoire complexity was further examined by the highly sensitive PCR-based complementarity-determining region 3 (CDR3) size spectratyping analysis, in which the bulk TCR sizes for any given Vβ family of a control population exhibit a Gaussian distribution ladder of in-frame expressible bands separated by three bases (19, 20). A skewing of the normal size distribution, reflected by increased band intensity, is indicative of an expanded CDR3 size expression and suggests an oligoclonal T cell response (21). Characterization of the TCR Vβ repertoire of the responding donor CD8+ T cells is the first step toward ultimate identification of the specific T cells involved in the GVHD response. By this approach, we have found that B6→BALB.B TDL T cells exhibited biased CDR3 size usage in the Vβ1, 4, 6, 8, 9, 10, and 14 families, whereas B6→CXBE TDL T cells exhibited overlapping usage of the Vβ1, 6, 8, 9, 10, and 14 families. The Vβ4 response appeared to be unique in recognition of miHA expressed only by BALB.B recipients. The implications of these findings are that GVHD in this model system seems to involve an oligoclonal response to a limited number of immunodominant miHA. Furthermore, these miHA appear capable of inducing T cell responses in both the CXBE RI and BALB.B parental strains, with no evidence of competitive inhibition as previously observed in vitro (14). In addition, CD8+ Vβ14+ B6 T cells displayed high GVHD potential upon transplantation into irradiated BALB.B and CXBE mice, supporting the involvement of T cells from an individual Vβ family in the development of GVHD.

**Materials and Methods**

**Mice**

C.B10-H2/2/LiMcJ (BALB.B), CBX-2/Bx (CXBE), and C57BL/6By (B6) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and/or raised in our breeding colony from breeder pairs provided by The Jackson Laboratory. For all experiments, sex-matched mice were used as donors and recipients between the ages of 7 and 16 wk. Mice were kept in a pathogen-free environment in autoclaved microisolator cages and were provided with acidified (pH 2.5) water and autoclaved food ad libitum. PBS (BioWhittaker, Walkerville, MD) supplemented with 0.1% BSA (Sigma Chemical, St. Louis, MO) was used for all in vitro manipulations of the donor bone marrow cells and lymphocytes. RPMI 1640 (Mediatech, Herndon, VA) supplemented with 10% FCS (Sigma), 2 mM l-glutamine (Mediatech), 50 IU/ml penicillin (Mediatech), 50 μg of streptomycin (Mediatech), and 5 IU/ml heparin sulfate (Schein Pharmaceutical, Florham Park, NJ) was used for TDL collection. PBS supplemented with 1% BSA and 0.1% NaN3 was used as a medium for staining cells for flow cytometry (FACS media). PBS alone and supplemented with 1% BSA was used during magnetic cell sorting.

**Media**

PBS was injected i.v. (in a volume of 0.5 ml of PBS) with the appropriate preparation (ATBM (2 x 10^7) or RPMI 1640 (2 ml)). ALF-D was added to the experimental cell preparation to be tested. Mice were checked daily for morbidity and mortality and weighed twice per week until the experiments were terminated. Statistical comparisons between groups were performed by the Wilcoxon nonparametric test.

**Thoracic duct cannulation**

Thoracic duct cannulation was used to obtain positively selected host miHA-specific T cells, as previously described (18, 25). BALB.B or CXBE mice were lethally irradiated (825 cGy) and ~6 h later injected i.v. with (2 x 10^7) ALF-D in RPMI 1640, and then euthanized by the Wilcoxon nonparametric test.

**Flow cytometric analysis of TCR Vβ expression**

For fluorescent staining, cells were washed, resuspended in FACS medium, and incubated for 5 min at 4°C with culture supernatant containing anti-FcγRII mAb (clone 2.4G2; HB97; Amgen, Thousand Oaks, CA) and anti-FcγRIII mAb (clone 7.23.1; Cappel-Organon Teknika, Westchester, PA). After washing three times, these cells were incubated with (2 x 10^7) ALF-D in RPMI 1640, washed twice, and resuspended in PBS and incubated with MAGS Magnetic streptavidin microbeads from Miltenyi Biotec, Bergisch Gladbach, Germany for 30 min at 4°C. The cells were washed twice, resuspended in PBS + 0.1% BSA, and positively selected by either fluorescent cell sorting using an EPICS ELITE flow cytometer (Coulter Electronics, Hialeah, FL) or the Vario MACS Column System (Miltenyi). CD8+ Vβ14+ T cells were washed twice and resuspended in PBS for i.v. injection. For the GVHD experiments with CXBE recipients, CD4+ T cells were not depleted from the donor cell populations.

**GVHD assay**

BALB.B or CXBE mice were lethally irradiated (825 cGy) and ~6 h later injected i.v. (in a volume of 0.5 ml of PBS) with the appropriate preparation (ATBM (2 x 10^7) or RPMI 1640 (2 ml)). ALF-D was added to the experimental cell preparation to be tested. Mice were checked daily for morbidity and mortality and weighed twice per week until the experiments were terminated. Statistical comparisons between groups were performed by the Wilcoxon nonparametric test.

**Irradiation**

Recipient mice received a lethal dose of whole body irradiation (825 cGy) from a GammaCell (Atomic Energy of Canada, Kanata, Ontario, Canada) 137Ce source at a dose rate of 116 cGy/min.

**Monoclonal antibodies**

Ascites fluid for anti-Thy-1.2 (J1, rat IgM (22), anti-CD4 (RL172, rat IgM (23), and anti-CD8 (clone 53-6.7; no. 01044D) mAb and biotinylated mAb specific for Vβ2, 3, 4, 5/1, 2, 6, 7, 8, 1/2, 9, 10, 11, 12, and 13.

**Preparation of donor cells**

Bone marrow cells were flushed from the femurs and tibiae of B6 donor mice with PBS + 0.1% BSA and washed. To prepare anti-T cell-depleted bone marrow (ATBM), cells were incubated with J1 mAb (1:100 dilution of ascites fluid) and guinea pig C (1:30 dilution) in 6 ml of PBS + 0.1% BSA at 37°C for 50 min, and washed three times. ATBM was adjusted to 1.6 x 10^7 cells/ml in PBS for i.v. injection (0.1 ml) into recipients. T cell-enriched donor cells were prepared from pooled spleen and lymph node cell suspensions from appropriate BALB.B or CXBE primed and boosted B6 mice (i.p. injection of 1.5 x 10^7 spleen cells 2.5 wk apart). The cells were washed and resuspended in Gey's balanced salt solution containing 0.7% NH4Cl to remove RBC. The cells were washed twice and filtered through a cell strainer to remove dead cells. B cells were removed by panning the cell suspension on goat anti-mouse IgG precoated plastic petri dishes for 1 h at 4°C, as previously described (16). The nonadherent (whole T cell enriched) cells were harvested. Cell purity was >95% positive for CD3 expression, as determined by flow cytometry. For induction of GVHD, whole T cells were depleted of CD4+ T cells by incubation with RL172 mAb (1:100 dilution of ascites fluid) and guinea pig C (1:30 dilution) in 6 ml of PBS + 0.1% BSA at 37°C for 50 min and washed three times. CD8+ enriched cells were incubated with either biotinylated or FITC-conjugated mAb specific for Vβ14 mAb was adjusted to 10^7 cells/ml in PBS for 30 min at 4°C. Cells were washed twice in PBS and incubated with MAGS Magnetic streptavidin microbeads from Miltenyi Biotec, Bergisch Gladbach, Germany for 30 min at 4°C. The cells were washed twice, resuspended in PBS + 0.1% BSA, and positively selected by either fluorescent cell sorting using an EPICS ELITE flow cytometer (Coulter Electronics, Hialeah, FL) or the Vario MACS Column System (Miltenyi). CD8+ Vβ14+ T cells were washed twice and resuspended in PBS for i.v. injection. For the GVHD experiments with CXBE recipients, CD4+ T cells were not depleted from the donor cell populations.
cells were assayed for the percentage of Vβ expressing CD8⁺ T cells by two-color flow cytometry. Cells were first incubated with each biotinylated anti-Vβ mAb in combination with FITC-anti-CD8 mAb for 30 min at 4°C. The cells were then washed twice and incubated with PE-streptavidin (CalTAG, San Francisco, CA) for 30 min at 4°C, washed twice, and fixed in 1% paraformaldehyde. Cells were analyzed on an EPICS C flow cytometer (Coulter Electronics). Data from individual replicate experiments were pooled, and statistical significance was determined by the Mann-Whitney Wilcoxon rank test.

Preparation of RNA and cDNA

CD8⁺ T cells were first prepared by depletion of CD4⁺ T cells via panning of TDL cell suspensions on GK1.5 mAb (1:20 dilution of ascitic fluid in PBS + 0.1% BSA) precoated plastic petri dishes for 1 h at 4°C. This procedure yielded a restricted population with ≥92% CD8⁺ cells. Total cellular RNA was then generated from 10⁶ to 10⁷ nonadherent CD8⁺ cells by homogenization in 1 ml of Ultraspec (Biotex Laboratories, Houston, TX), separating cellular DNA and protein by the addition of a 1.5 volume of chloroform, vortexing for 5 s, and centrifuging at 12,500 rpm for 15 min. The aqueous phase was transferred to a clean Eppendorf tube, and RNA was precipitated at 4°C by adding an equal volume of isopropanol and centrifuging for 15 min. The supernatant was removed, and the pellet was washed with 75% ethanol in diethyl pyrocarbonate (DEPC) treated water and centrifuged, as above. The RNA pellet was resuspended in 25 μl of DEPC water, heated to 55–65°C for 10 min and stored at −20°C. Recovery of RNA was determined by spectrophotometry. The poly(A)⁺ portion of the total RNA was converted into cDNA using oligo(dT) as a primer for reverse transcription. Two micrograms of total RNA in a volume of 9.5 μl were heated to 70–80°C, centrifuged briefly, and placed on ice. Master mix (17.5 μl), containing 1 μl of RNasin (40 U/μl), 6 μl of 5X Maloney murine leukemia virus reverse transcriptase reaction buffer, 3 μl of oligo(dT) primer (20 mM), 1.5 μl of deoxynucleotide triphosphates A, G, C, T (25 mM each), and 3 μl of Maloney murine leukemia virus reverse transcriptase (300 U/μl), was incubated at 37°C for 1.5 h to synthesize cDNA. All reagents were purchased from Promega (Madison, WI). The reaction mixture was then heated to 95°C for 3 min, and the cDNA was stored at −20°C. PCR was performed using murine β-actin primers to establish the quality of the cDNA.

PCR amplification of cDNA and CDR3 size spectratyping

PCR was performed using a labeled constant primer (Cβb) and a Vβ primer specific for each Vβ family to be analyzed. Cβb was labeled using polynucleotide kinase (Promega) and [γ-32P]ATP (DuPont-NEN, Boston, MA). All the primers used have been previously described (27) with the exception of the Vβ20 primer, which was designed with the sequence of GGTCAAGGAGAGATTCTCAGCTGT. Five microliters of 10³ Tαγ polymerase buffer B (Promega) were added to 5 m, 28 μl DEPC-treated water, and 1 μl of Taq polymerase (2.5 U/ml, Promega). Thirty cycles of amplification were conducted and on completion of PCR, 50 μl of 2X loading buffer containing 95% formamide, 20 mM EDTA, 0.05% bromphenol blue, and 0.05% Xylene Cyanol FF was added to each reaction. PCR reactions were then either stored at −20°C or electrophoresed. Each reaction tube was heated to 70°C for 3 min, and 6 μl of each were applied to a prewarmed 6% acrylamide sequencing gel, as previously described (20). The gels were run at 55 mA for 1.5 h to maximize band resolution. Sequencing gels were dried, and autoradiography was generally performed for 15 h at room temperature without intensifying screens. Densitometric scanning of autoradiographs was performed on a Personal Densitometer SI using ImageQuant software (Molecular Dynamics, Sunnyvale, CA). The interpretation of the spectratypes were based on the criteria previously established by Gorski et al. (19, 20). The relative band intensities within a given spectratype were examined and compared with the intensity patterns in the spectratype of the naive B6 control group. The other bands within a given spectratype act as the internal controls for any variability due to the PCR expansion or to different sample sizes.

Results

General approach

B6 T cells (1.5 × 10⁷) were injected into irradiated (825 cGy) BALB.B and CXB6 mice for induction of GVHD. It was necessary to use cells from donor mice that had been presensitized (primed and boosted by i.p. injection of 1.5 × 10⁷ spleen cells) to the appropriate recipient type to generate any significant TCR Vβ family expansion of the CD8⁺ T cell population within the first week posttransplantation. The recipients underwent thoracic duct cannulation on day 5, and TDL was collected over a 20-h period and pooled from three to four mice per group. The positively selected TDL were >95% CD₃⁺ and consisted of 55 to 67% CD₄⁺ and 33 to 45% CD₈⁺ T cells. A significant percentage (20–25%) of the TDL were blast-like in size, in contrast to TDL collected from B6 mice transplanted with T cells (1–1.5 × 10⁷) from syngeneic-presensitized B6 mice, which yielded few blast-like cells and were >98% CD₄⁺. Therefore, the TCR Vβ repertoire analyses of TDL CD8⁺ T cells from GVHD recipients were compared with the TCR Vβ repertoire of normal B6 splenic CD8⁺ T cells.

TCR Vβ repertoire analysis by flow cytometry

TCR Vβ repertoire analysis was conducted by two-color flow cytometry of the TDL using a panel of anti-Vβ mAb along with anti-CD8 mAb. Of the 13 Vβ families for which specific mAb were available, positively selected B6→BALB.B TDL did not display any significant Vβ family expansion (Fig. 1). On the other hand, the B6→CXBE TDL exhibited significant expansions of the CD8⁺ T cell population within the first week posttransplantation. The recipients underwent thoracic duct cannulation on day 5, and TDL was collected over a 20-h period and pooled from three to four mice per group. The positively selected TDL were >95% CD₃⁺ and consisted of 55 to 67% CD₄⁺ and 33 to 45% CD₈⁺ T cells. A significant percentage (20–25%) of the TDL were blast-like in size, in contrast to TDL collected from B6 mice transplanted with T cells (1–1.5 × 10⁷) from syngeneic-presensitized B6 mice, which yielded few blast-like cells and were >98% CD₄⁺. Therefore, the TCR Vβ repertoire analyses of TDL CD8⁺ T cells from GVHD recipients were compared with the TCR Vβ repertoire of normal B6 splenic CD8⁺ T cells.
family, additional bands were enhanced in the B6→BALB.B CD8⁺ TDL spectratype which were not skewed in the B6→CXBE CD8⁺. Most notably, biased CDR3 usage in the Vβ4 family was unique to the B6→BALB.B CD8⁺ TDL, suggesting a response to a BALB.B-specific miHA.

**Induction of GVHD by T cells from a single TCR Vβ family**

Fluorescent cell sorting was used to positively select CD8⁺ Vβ14⁺ T cells from CXBE-presensitized B6 mice which were then transplanted into irradiated (825 cGy) BALB.B recipients to demonstrate that cells from a single Vβ family were capable of inducing GVHD. Recipients were injected with either the combination of 3× 10⁶ naive B6 CD4⁺ T cells (to provide Th function) and 2× 10⁶ ATBM alone or with either 4× 10⁶ CXBE-presensitized unseparated B6 CD8⁺ T cells or 1× 10⁵ CD8⁺ Vβ14⁻/-enriched T cells (>99% Vβ14⁺). Recipients of either the unseparated or Vβ14⁺ CD8⁺ T cells exhibited the clinical symptoms of GVHD between days 45 and 50, including weight loss (22 and 30%, respectively; Fig. 4A). By day 76, 60% (MST of 49 days) of the BALB.B mice that received the unseparated CD8⁺ T cells and 80% (MST of 67 days) of those given the Vβ14⁺ CD8⁺ T cells had died, compared with 20% mortality in the ATBM control group (Fig. 4B). In a similar manner, lethally irradiated (825 Gy) CXBE mice were injected with 2× 10⁶ ATBM either alone or with either 3× 10⁷ CXBE-presensitized unseparated or 3.5× 10⁶ Vβ14⁺ enriched (>84% Vβ14⁺ selected by magnetic cell sorting) B6 T cells. Both groups of CXBE recipients that received T cells exhibited the clinical symptoms of GVHD by day 40, including weight loss (17 and 25%, respectively; Fig. 5A). By day 45, mice that received injections of Vβ14⁺-enriched CD8⁺ T cells had 80% fatality (MST of 48 days), and by day 70, the mice that had been injected with unseparated CD8⁺ T cells had 40% fatality, as compared with 100% survival in the control ATBM group (Fig. 5B).

**Discussion**

The TCR Vβ repertoires of positively selected TDL collected from presensitized B6→BALB.B and B6→CXBE transplanted mice were analyzed to gain insight into the CD8⁺ T cell populations that mediate GVHD directed against multiple miHA disparities. The TDL pool is an excellent site for monitoring T cell responses since recently activated cells expand and enter the thoracic duct after retrieval of T cells from the peripheral lymphoid organs (28). Due to the overall low frequency responses involved in anti-miHA reactivity and the slower development of primary CD8⁺ T cell responses in vivo relative to CD4⁺ T cells, significant phenotypic skewing of Vβ families was not detectable over a period of 3 to 8 days posttransplantation of naive B6 T cells, although significant skewing occurred in the CD4⁺ T cell population (18). The cannulation of mice at later time points was impractical, since the mice began developing GVHD-related symptoms and could not survive the procedure. As an alternative, anti-host miHA T cells were first expanded by presensitization and boosting of donor B6 mice in vivo with host-type splenocytes. We then investigated the response patterns of these donor cells when placed in a GVHD-inducing environment in the irradiated BALB.B or CXBE recipients. To confirm that the TDL contained alloreactive anti-miHA-specific T cells, TDL collected from the B6→BALB.B combination were injected into lethally irradiated BALB.B and CXBE recipients. The ability of these cells to induce GVHD in both hosts suggested that the TDL contained alloreactive CD8⁺ T
cells. Lethal GVHD in the B6→BALB.B model is mediated by either CD4⁺ or CD4-dependent CD8⁺ T cells, while in the B6→CXBE model only CD4-dependent CD8⁺ T cells are responsible for pathogenesis (17). The phenotypic analysis was not sensitive enough to detect any major shifts in the B6→BALB.B TDL but did indicate expansions of at least the Vᵦ₁₀ and Vᵦ₁₄ families in the B6→CXBE TDL (Fig. 1). This observation was consistent with the involvement of a limited number of distinct mHA epitopes in the development of the B6 CD8⁺ anti-CXBE GVHD response. It was not overly surprising that phenotypic analysis of the B6→BALB.B TDL failed to detect a specific Vᵦ family response. This approach is capable of detecting only major shifts in Vᵦ utilization, and even if successful, it is unable to further characterize the response and determine whether it is oligoclonal. A phenotypic increase in Vᵦ utilization could also be due to polyclonal expansion which would represent increased expression of many TCR V-D-J sequences within that Vᵦ family. For example, polyclonal expansion was previously observed for the CD4⁺ Vᵦ₃ family in the B6→BALB.B strain combination and was likely due to MTV-6 superantigen stimulation (18). T cell repertoire analysis based on the size heterogeneity of the CDR3 region is, therefore, a much more sensitive and powerful tool for the study of GVHD and other types of immune responses (21, 29, 30). It also provides a mechanism for differentiating the specific TCR V-D-J sequences expressed within the same Vᵦ family, and can reveal much more subtle skewing within a Vᵦ family than can be detected by FACS analysis.

are the spectratypes of Vᵦ₁₀, 14, 4, and 5. B. Densitometric scanning of the gel films in A generated histograms to compare the size distribution of CDR3 bands between the B6 naive splenic T cells (histogram 1), and B6→CXBE (histogram 2) or B6→BALB.B TDL (histogram 3). Arrows indicate CDR3 bands that display increased relative intensities within the spectratype. Starred peaks in the Vᵦ₁₄ histograms are contaminants and not part of the spectratype.
The increased Vβ heterogeneity observed in the CDR3 size spectratyping analysis would suggest that GVHD is mediated by a heterogeneous population of alloreactive CD8+ T cells that recognize either the same miHA, several miHA, or multiple epitopes of the same Ag in the host. Yet, the overall responses to miHA in the B6→BALB.B and B6→CXBE strain combinations were limited to only a few Vβ families, supporting the notion that a limited number of miHA or epitopes were being recognized. The involvement of a limited number of immunodominant miHA in GVHD has been previously found by Perreault et al. in another model system (31). In addition, expansion of a limited set of CD8+ Vβ families in local liver GVHD pathogenesis has been observed by Howell et al. (32). It has also been shown that Ag-specific CD8+ T cells exhibit limited heterogeneity at the level of TCR Vβ gene usage during a primary response to HIV infection (33). The notion of heterogeneous Vβ responses developing against a single or limited number of miHA is highly possible since T cell recognition of Ag involves the interaction of both Vβ and Vα chains (34). Each of the responding Vβ chains could associate with a different Vα chain to recognize the same epitope. This has been observed in the diversity of CD4+ T cell responses to influenza antigenic determinants (35).

It is estimated that more than 29 miHA locus differences exist between the B6 and BALB/c (and therefore the BALB.B) inbred mouse strains (36). Yet only a few immunodominant miHA appear to be recognized in vivo by MHC-matched allogeneic T cells responsible for skin graft rejection (37). An immunodominant hierarchy was also found to operate for the generation of miHA-specific CTL in vitro (14) and was possibly due to the comparative abilities of miHA to compete successfully for Ag-binding residues in the appropriate MHC molecules presented by APC. The affinity/avidity of the interaction of the specific TCR for miHA/self-MHC may also be an important factor of miHA immunodominance. Alternatively, T cells that respond more vigorously to strong miHA may down-regulate weaker responses to other miHA via cytokine production. Each of these potential mechanisms or combinations thereof could account for the phenomenon of competitive immunodominance. Recent studies comparing CTL immunodominant specificities and skin graft rejection in the B6 anti-BALB.B and CXB strain combinations have found a distinct lack of correlation between the two responses, with CTL recognizing only a limited number of miHA operative in vivo (13).

A similar lack of correlation with CTL immunodominant specificities had previously been found in GVHD (15, 17). For GVHD then, the relevant question becomes how extensive are the number of miHA operative in vivo (13). At least two of these criteria would seem to be immunogenicity and tissue distribution. In terms of the latter, it is known that some miHA have unique tissue expression (38, 39), and it would be expected that for optimum GVHD pathogenesis, Ag should be available not only in cells of hemopoietic origin but also in the primary target organs, including the intestine, liver, and skin. The lack of GVHD in irradiation chimeric models that have
miHA expressed only in the host hemopoietic compartment supports the notion that the presence of miHA in both locations is required. Thus, there may only be a limited number of miHA that fit the criteria for GVHD. In this regard a recent study of the CBX interstrain lethal GVHD responses suggested the minimal involvement of two distinct immunodominant miHA (or associated groups of miHA) in the B6→BALB.B GVHD response (16). One class I-restricted miHA (GVH-1) appeared to be shared by the CBX strain, while the second Ag (GVH-2) was uniquely expressed by the BALB.B strain. The biased CDR3-size skewing of Vβ1, 6, 8, 9, 10, and 14 families in both the BALB.B and CBX recipients may represent a response to common miHAs shared by BALB.B and CBX mice, i.e., GVH-1. Although the same CDR3 size bands were skewed in each corresponding Vβ family (except for Vβ14), the final identity of these specificities will depend on sequence analysis of the CDR3 segments involved in each response. Furthermore, the biased CDR3 usage of Vβ4 in the BALB.B recipients, not present in the spectratype of the CBX recipients, might also represent a response to the unique BALB.B miHA, i.e., GVH-2. Experiments are under way to determine whether positively selected CD8+ Vβ4+ T cells will induce GVHD in BALB.B but not CBX recipients. The biased CDR3 size skewing of Vβ14 displayed the only nonidentical skewing pattern between the B6→BALB.B and B6→CBX T cell responses. The BALB.B spectratype exhibited two additional skewed bands. The additional bands present in the Vβ14 spectratype of the BALB.B recipients might also represent a response to the unique BALB.B miHA (GVH-2). Although B6 Vβ14+ T cells injected into either BALB.B or CBX recipients induced lethal GVHD (80% mortality by day 80 for both groups of recipients; Figs. 4B and 5B), the number of cells injected into the BALB.B recipients was 30-fold less (1 × 105 vs 3.3 × 106) than into the CBX recipients. This difference in the response suggested a possible additive effect of different anti-miHA clonotypes in the BALB.B, consistent with GVH-1 and GVH-2. The capacity of presensitized Vβ14+ donor T cells to cause lethal GVHD is noteworthy on its own accord. It has been very difficult in the past to demonstrate GVHD induction with normal donor T cells directed to limited miHA specificities. The use of miHA congenic strain combinations has failed to generate detectable GVHD, even when donor cells were presensitized to host Ags (16, 41). Single cloned T cells have been used successfully before to induce GVHD (42), but these were CD4+ T cells. Furthermore, any clone (CD4+ or CD8+), due to their extensive expansion in culture, could potentially differ from normal T cells in mediation of disease pathogenesis (e.g., by abnormal acquisition or loss of adhesion molecules responsible for migration into target tissue). The use of normal donor T cells with limited heterogeneity to generate GVHD, as with the Vβ14+ cells, will ultimately enable the investigation of associations between specific anti-miHA responses and tissue distribution of lesions.

Characterization of the TCR usage by T cells responding to miHA and mediating GVHD will help us to understand the scope of these complex responses and ultimately the nature of the miHA responsible for their induction. With future developments in diagnostic capabilities, our appreciation for the GVHD response repertoire may also eventually allow for new targeted strategies for prevention of GVHD.

Acknowledgments

We thank David Dicker for his expert technical assistance in performing the flow cytometric analysis and fluorescent cell sorting, as part of the Kimmel Cancer Institute Flow Cytometry Facility.

References