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Research Article

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Specificity of T Cells Invading the Skin during Acute Graft-vs.-Host Disease after Semiallogeneic Bone Marrow Transplantation

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Abstract

The mechanisms responsible for skin lesions during acute graft-vs.-host disease (aGVHD) after allogeneic bone marrow transplantation (BMT) are poorly understood. The exact role of various effector cell populations and “major” (particularly HLA-DP) or “minor” antigens as target molecules is not known. To investigate the nature of cells responsible for tissue injury, we cultured T cells from skin biopsy first with interleukin 2 (IL-2) alone and then in polyclonal activation conditions to avoid in vitro antigenic sensitization before specificity testing. We applied this method to two biopsies performed during aGVHD after semiallogeneic BMT and obtained cytotoxic T cells against four graft mismatches: CD8⁺ T cells against HLA-A2.2 and HLA-B27 and CD4⁺ T cells against HLA-DP101 and HLA-DP401. This demonstrates that T cells with documented specificity can be obtained from an aGVHD lesion without antigenic selection. Moreover, these data directly implicate DP as a potential target antigen for aGVHD. (*J. Clin. Invest.* 1993. 91:12–20.) Key words: alloresponse • clones • engraftment • HLA-DP • mismatch

Introduction

Although T cells have not been directly implicated in the etiology of acute graft-vs.-host disease (aGVHD)¹ in humans, their importance in this process is strongly suggested by the following observations: T cell depletion is efficient in preventing aGVHD (1–4); a correlation exists between the number of engrafted T cells and the severity of aGVHD (5–9), as well as between anti-host-cytotoxic T lymphocyte precursor frequency (pCTL) and the occurrence of aGVHD (10); and T cells are present at the site of aGVHD lesions (11–16). However, the nature of the molecular and cellular targets and the effector lymphocytes responsible for target damage remain to be determined. One interesting feature of GVHD is the specificity of the lesion site (mainly digestive epithelia and epidermis),

in that the mechanisms by which nonlymphoid target tissues are selected for immune-mediated damage are poorly understood. The fact that T cells can distinguish between allogeneic major histocompatibility complex products on different cell types (17), and that in GVHD a high number of nonepithelial cells expressing recipient class II antigens in many tissues are not affected by GVHD (18), raises the question of T-target interaction during GVHD, either through “minor” or “major” antigenic disparities. However, as tissue specificity is always observed regardless of the genetic combination, it is likely that allorecognition through the T cell receptor cannot fully account for epithelium targeting.

Goulmy and co-workers have documented the presence of anti-host-specific CTL among recipient peripheral blood lymphocytes (PBL). These CTL, probably directed at minor antigens, can lyse host PBL isolated before grafting (19). However, the same group has also detected these CTL in PBL of patients with no clinical signs of GVHD (20). This latter observation suggests that PBL studies, particularly when performed after several in vitro stimulations with host cells, do not reflect what takes place at a GVHD lesion site. Other authors have obtained T cells from skin lesions either by stimulation at the beginning of the culture with allogeneic B lymphoblastoid cell lines (BLCL) sharing HLA antigens with host cells (21) or, after an initial culture period with IL2 alone, by a coculture with host BLCL before testing (22). In both cases, anti-host cells were obtained, but no antigenic specificity could be defined in these HLA-identical situations.

Our goal in this work was to characterize T cells from a GVHD lesion site which were only sensitized in vivo against the host. We therefore investigated lymphoid populations infiltrating the skin from two patients during aGVHD after allogeneic haploidentical bone marrow transplantation (BMT). A specific culture protocol was used to avoid any anti-host in vitro sensitization. Our results demonstrate that cell lines and clones thus obtained were specific for two mismatched HLA antigens between donor and recipient in both cases: HLA-A2 and DP1 for patient 1, and HLA-B27 and DP4 for patient 2. These data document the presence of T cells of known specificity (involving HLA-DP) with cytotoxic potential at a GVHD lesion site. These investigations also provided us with cellular material of potential use for further studies on the molecular structures recognized in vivo. The fact that anti-class II T lymphocytes are obtained when class II bearing Langerhans cells are rare or absent from the skin is discussed, as well as the implication of T cell receptor-antigen interaction in directing tissue specificity during GVHD.

Methods

Patients

Patient 1. A 9-yr-old boy was grafted for chronic myelogenous leukemia with his mother's bone marrow. HLA typing is indicated in Table I

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1. *Abbreviations used in this paper:* aGVHD, acute graft-vs.-host disease; BLCL, B lymphoblastoid cell lines; BMT, bone marrow transplantation; BRMP, Biological Response Modifier Program; CsA, cyclosporin A; SBD, skin biopsy-derived; TBI, total body irradiation; TCR, T cell receptor.

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Table I. HLA Typing of the Two Hosts and Patients and Panel B Lymphoblastoid Cell Lines (BLCL)

Code	BLCL	Sex	HLA-A	HLA-B	HLA-DR	HLA-DQ	HLA-DP β
A	AMALA	F	2.4	62.3	14	7	0402
B	BH	F	2.2	13.2	7	2	0402
C	BM16	F	2.2	18.1	12	7	0202
D	BSM	F	2.2	62.3	4	8	0202
E	BOLETH	M	2.2	62.3	4	8	0401
F	CALOGERO	M	2.2	61	16	5	0402
G	DEM		2.2	57	16/4	5/8	0301/0401
H	DUCAF	M	30.1	18.1	3	2	0202
I	E4181234	M	1	52	15	6	0202/0402
J	IBW9	M	33.1	65	7	2	0101
K	JVM	M	2.2	18.1	11	7	0201
L	OMW	M	2	45	13	6	0101
M	RML	M	2.4	51	16	7	0402
N	RSH	M	68.1/30.3	42	3	4	0101/0402
O	SPOO10	F	2.2	44.2	11	5	0201
P	VAVY	F	1	8	3	2	0101
Q	WT24	M	2.2	27.5	16	5	0301
R	BM14	M	3	7	4	8	0401
S	HOM2	F	3	27	1	5	0401
T	BTB	M	2	27	8	4	0401
V	MGAR	F	26	8	15	6	0401
	Host 1	M	1/2	7/8	3/6	2/13	0101/19
	Donor 1	F	1/1	7/8	3/6	2/13	0301/19
	Host 2	M	9(24)/32	27/17	4(14)/8	8/4	401/1001
	Donor 2	F	9(24)/32	8/17	3/8	2/4	601/1001

Alleles are indicated only once for homozygous BLCL.

(the DP mismatch was not known before grafting). The conditioning regimen consisted of cyclophosphamide (120 mg/kg) and total body irradiation (TBI): 12 Gy through six irradiation courses. GVHD prophylaxis consisted of cyclosporin A (CsA) and methotrexate (at days 1, 3, 6, and 11) and 5 mg/day of BB10, an anti-IL-2R antibody, for 10 d. GVHD was suspected at day 34, and three skin biopsies were performed for histological study, immunohistochemistry, and culture. A large infiltrate was observed, composed of 70% CD8⁺ and 5% CD4⁺ T cells, whereas no CD1⁺ Langerhans cells were detected. Keratinocytes expressed class II. Biopsy-confirmed aGVHD was treated with CsA (3 mg/kg·d), corticosteroids (2 mg/kg·d), and BB10. Unfortunately, treatment failed and GVHD extended to gut and liver. The patient died at day 99 after grafting.

Patient 2. A 7-yr-old boy was grafted with his mother's bone marrow for acute myelogenous leukemia in the second complete response. HLA typing is indicated in Table I. The conditioning regimen consisted of TBI and high doses of cytarabine and melphalan. T cell depletion was performed as GVHD prophylaxis using monoclonal antibody (mAb) anti-CD2, anti-CD7, and rabbit complement. In addition, the patient received anti-LFA1 and anti-CD2 mAbs (0.2 mg/kg·d) from day -3 to day 12. GVHD was suspected at day 19 after grafting. The biopsy was not immunologically informative. It showed a moderate infiltrate with a few CD4⁺ cells and no detectable CD8⁺ as well as rare Langerhans cells; keratinocytes did not express class II. This immunological picture could correspond to the beginning of the GVHD process, and in fact GVHD was histologically and clinically confirmed and then treated by corticosteroids (2 mg/kg·d) and CsA (3 mg/kg·d). Worsening of skin GVHD on day 7 prompted us to start treatment with anti-TNFA α (5 mg/d 4 d) in association with anti-CD25 (5 mg/d for 10 d and then 5 mg every other day for 40 d). Skin GVHD resolved after 4 d and no chronic GVHD was documented.

This patient relapsed on day 120 after transplantation, and is currently receiving chemotherapy.

Materials and methods

Expansion of T lymphocytes from skin biopsy: skin specimens were washed three times in RPMI containing 10% pooled human sera, 1% L-glutamine (2 mM), and 50 μ g/ml gentamycin and then cultured in a 24-well plate (Nunc, Copenhagen, Denmark) in the same medium supplemented with recombinant interleukin 2 (rIL-2) (150 Biological Response Modifier Program (BRMP) U/ml; rIL-2 generously provided by Dr D. Lando from Roussel-Uclaff, Romainville, France). Cultures were kept at 37°C in a 5% CO₂ atmosphere. After 2 wk in culture with IL-2 alone, the cell lines stopped proliferating. To further expand these populations, 10³ T cells per well were seeded in a 96-microwell round-bottomed culture plate together with 5 \times 10⁴ allogeneic PBL and 5 \times 10³ allogeneic BLCL (3,000 rads irradiated) in the presence of leucoagglutinin-A (1 μ g/ml) (Pharmacia, Uppsala, Sweden), indomethacin (1 μ g/ml), and rIL-2 (150 BRMP U/ml). These conditions, allowing maximal cloning efficiency for T cells (as assessed by limiting dilution analysis: frequency of proliferation about 1 in 2) were chosen to avoid in vitro selection, while maintaining as nearly as possible the best representation of the T cell population which grew under IL-2 alone during the first culture period. These same conditions were also used to generate a panel of clones by seeding one responder in every three culture wells. Before specificity assays, the cell line or the clones were cultured without stimulation in IL-2 or IL-4 (20 ng/ml) for 7–10 d.

Monoclonal antibodies

Phenotypic analysis was performed by indirect immunofluorescence using mAbs anti-CD2, -CD3, -CD4, -CD8, -TCR- α/β , and -TCR- γ/δ from Becton Dickinson, Grenoble, France; and anti-A2 (HB54

and HB117) and anti-B27 (HB157) from the American Type Culture Collection, Rockville, MD. The following mAbs were used for specificity studies: anti-DP/DR (2D6), -DP (B7.21), -DQ (1A3 and Leu-10), -DR (GSP41), -class I (W632), and class -II (TU39) from the 1987 HLA workshop.

Immunophenotyping

Cells (5×10^5 per mAb) were incubated for 45 min on ice with the indicated mAb at optimal concentration, washed three times in phosphate-buffered saline + bovine serum albumin (PBS-BSA 0.1%), then incubated for 45 min with fluorescein isothiocyanate (FITC)-labeled F(ab)₂ goat anti-mouse IgG (Immunotech, Marseille, France), washed again three times and fixed in PBS with 0.37% formaldehyde. An irrelevant anti-rat κ chain (MARC1) mAb was used as negative control.

Punch biopsies (4 mm) were taken and frozen in liquid nitrogen. Sections (3 μ m) were cut and immersed for 10 min in acetone and then washed in PBS for 10 min. After 30 min of incubation at 37°C with the appropriate mAb dilution, sections were washed in PBS. Fluorescein-conjugated goat anti-mouse IgG (Bio-Atlantique, Nantes) diluted 1/5 was then applied for 30 min at 37°C followed by washing in PBS. Sections were examined at $\times 320$ using a Zeiss microscope. Percentages were determined among 100 propidium iodide-positive cells in at least three different fields.

Cytotoxic assay

Cytotoxic activity was tested using a standard ⁵¹Cr release assay: Target cells were labeled with 100 μ Ci ⁵¹Cr for 2 h at 37°C, washed three times, and then plated at the indicated effector/target cell ratio in a 96-well round-bottomed plate. After 4 h of incubation at 37°C, 100 μ l of supernatant from each well was removed and counted in a gamma scintillation counter. Each test was performed in triplicate. Results are expressed as percentage of lysis, according to the following formula: (experimental release - spontaneous release)/(maximal release - spontaneous release) \times 100, where experimental release represents mean counts per minute released from the target cell in the presence of effector cells, spontaneous release that from target incubated without effectors and maximal release that from target incubated with 1% ce-tavlon.

Proliferation assay

10^4 resting T cells were cocultured for 4 d with irradiated (3,000 rads) BLCL in 96-microwell flat-bottomed culture plates at a 1:5 responder to stimulator ratio. 6 h before harvesting, 1 μ Ci of [³H]thymidine was

added to each well. [³H]thymidine uptake was measured in a liquid scintillation counter. Results are expressed as the mean of triplicate or quadruplicate cultures.

Southern blot analysis

Filter hybridization. After digestion with Eco RI or Hind III, 15 μ g of genomic DNA samples were size-fractionated by electrophoresis through 1% agarose gels. Southern blotting was performed using a vacuum blotting system (LKB-Pharmacia, St. Quentin en Yvelines, France) on Hybond N+ filter (Amersham, Les Ullis, France) according to the supplier's instructions. Hybridization, washing, and autoradiographies were performed as previously described (23) with ³²P multiprimed probe.

Probes. The J γ probe, pH60, containing the 700 base-pair HindIII-EcoRI fragment from M13H60, includes the J γ 1 segment (24, 25). This fragment cross-hybridizes with J γ 2 but not with JP, JP1, or JP2. The C β probe 1B10BB1 corresponds to the C β 1 fragment and cross-hybridizes with C β 2 (26).

Results

Ten biopsies from different clinical situations were studied. T cells could be obtained only when GVHD was confirmed histologically and clinically, strongly suggesting that they were able to grow in vitro in our culture conditions only when preactivated in vivo. In the two cases presented, T cell proliferation started as early as 3 d after initiation of the culture.

Patient 1

I. Generation of the skin biopsy-derived (SBD) T cell line. As only the recipient was HLA-A2⁺, HLA-A2-specific mAbs were used to determine whether SBD-T cells were of donor origin, i.e., HLA-A2⁻ (Fig. 1). At day 13, the SBD-T cell line consisted mostly of CD4⁺, TCR α/β ⁺ T cells. This was in sharp contrast with phenotyping results obtained from a contiguous biopsy, indicating that most (70%) infiltrating lymphocytes were CD8⁺ (data not shown). This suggested that CD8⁺ cells were rapidly overgrown by CD4⁺ cells during the bulk culture period.

II. SBD T cell line specificity. Initial testing of this bulk culture demonstrated both specific proliferation and cytotoxicity against host BLCL but no lymphokine-activated killer activ-

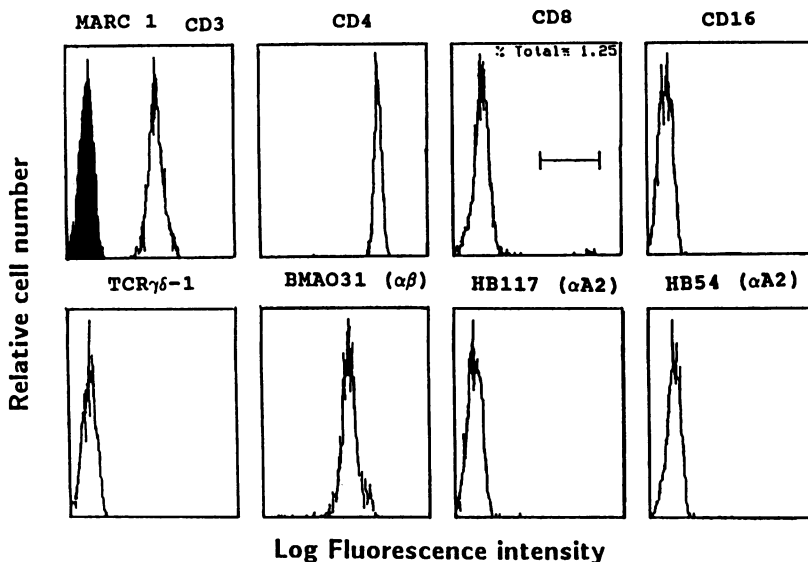


Figure 1. Phenotyping of the SBD T cell line. The negative control performed with the Marc-1 antibody (an anti-rat Ig- κ chain) is indicated in black on the first histogram, together with CD3.

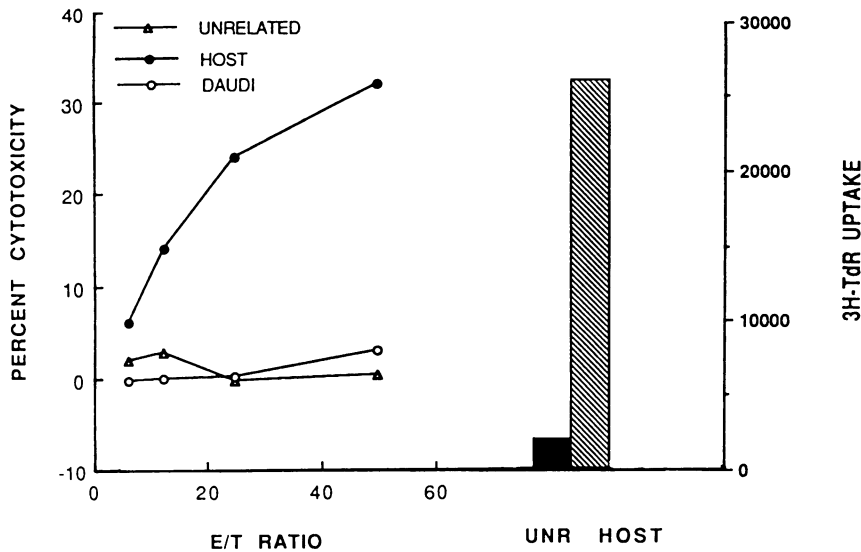


Figure 2. Specific recognition of donor BLCL by the SBD T cell line. Cell line cytotoxic activity was tested by a standard chromium release assay. Its proliferative activity was assessed after 72-h coculture with stimulator BLCL. This experiment is representative of 11 in which 18 unrelated BLCL were tested.

ity against the DAUDI cell line (Fig. 2). When antibodies against class I (W632), class II (TU39), DR (GSP41), DQ (Leu-10), and DP (B7.21) were added to the assay, proliferation was abrogated by TU39 and B7.21 mAb only (Fig. 3). Cell line specificity was then studied using the BLCL panel described in Table I. When tested for cytotoxic activity against 14 BLCL bearing different HLA antigens, the SBD-cell line demonstrated its cytotoxic potential only against host (HLA-DP101, 1901), J (BM9: HLA-DP101, 101), and P (VAVY: HLA-DP101, 101) BLCL (Fig. 4, top). No significant cytotoxicity was observed against any other BLCL tested. Specific recognition of HLA-DP101⁺ BLCL by the SBD T-cell line was confirmed by proliferation assays using four HLA-DP101⁺ and 4 HLA-DP101⁻ stimulator cells (Fig. 4, bottom).

III. Analysis of cell-line polyclonality and T cell clone specificity. The SBD T cell line was cloned by limiting dilution under nonspecific stimulatory conditions (see subsection *Materials and methods*).

T cell receptor (TCR) gene rearrangements of 34 T cell clones obtained were then analyzed by Southern-blot analysis using the TCR $C\beta 1/C\beta 2$ probe pC β and the $J\gamma$ probe pH60. As shown in Fig. 5, 11 different patterns were observed from combined analysis of Eco RI- and Hind III-digested DNA using the TCR β - and γ -specific probes, thus demonstrating the initial polyclonality of the cell line obtained. In agreement with results of SBD T cell line phenotyping, only one CD8⁺ clone was obtained; the others were CD4⁺ (data not shown). Two out of four different CD4⁺ T cell clones tested for proliferation against host and two panel BLCL demonstrated a strong and specific response against DP101-bearing target cells (Fig. 6). The only CD8⁺ T cell clone obtained (M15) showed a low but specific ability to proliferate against host cells (A1, A2) and BLCL G (DEM: A2.2, A2.2) but not against J, P, and I BLCL bearing non-A2 HLA antigens. BLCL A (AMALA: A2.4, A2.4) was not recognized (Fig. 7, top). Using a cytotoxic assay and a larger panel, we confirmed that clone M15 was specificity for all but one of the A2.2-bearing target cells (eight out of nine), but none of the others, including two A2.4 homozygous BLCL (Fig. 7, bottom). Among the seven HLA-A2 BLCL recognized for which sex origin was determined, four (K, Q, F, E) were of male and three (C, B, O) of female origin, demonstrating that this CD8 clone was not restricted to male HLA-A2. Taken together, these results demonstrate that clone M15 recognized an epitope on some HLA-A2 molecules, regardless of sex origin.

Patient 2

Because donor and recipient differed at HLA-B27 for which mAbs are available, we could also document that SBD-T cells in this case were all of donor origin (data not shown). Within the early bulk culture, 15% of CD8⁺ T cells were present although none were detected in a contiguous biopsy. These CD8⁺ cells were overgrown later on by CD4⁺ cells, as in the previous case (data not shown). Here again, this stresses the importance of rapid cloning of the culture.

SBD T cell line and clone specificity

Reactivity of the SBD-T cells derived from patient 2 against the mismatched allele HLA-B27 was already detectable in early

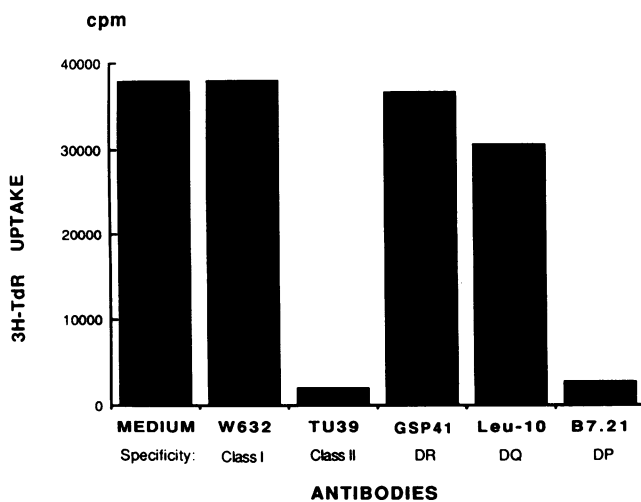


Figure 3. Recognition of target cells by the SBD T cell line is blocked by HLA class II- and HLA-DP-specific mAb. Cell line proliferative activity was assessed against a stimulatory BLCL in the presence of HLA-specific mAbs. Culture conditions were the same as for Fig. 2.

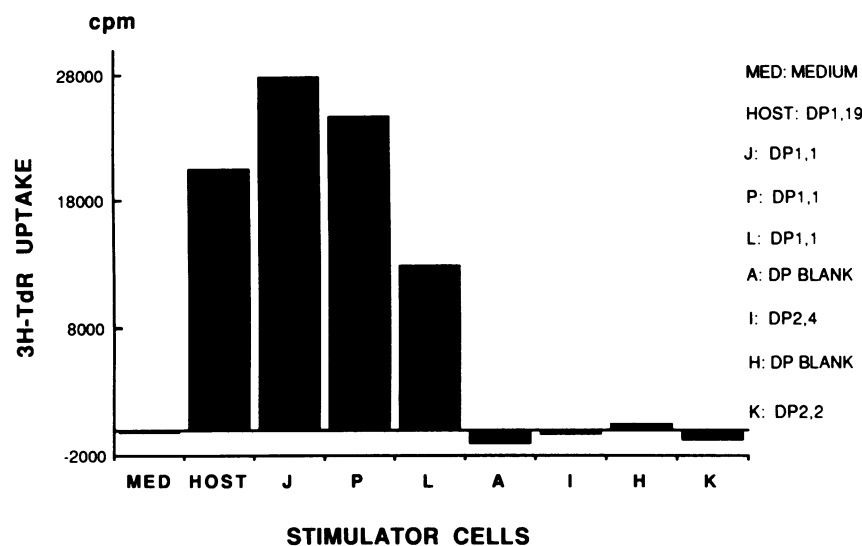
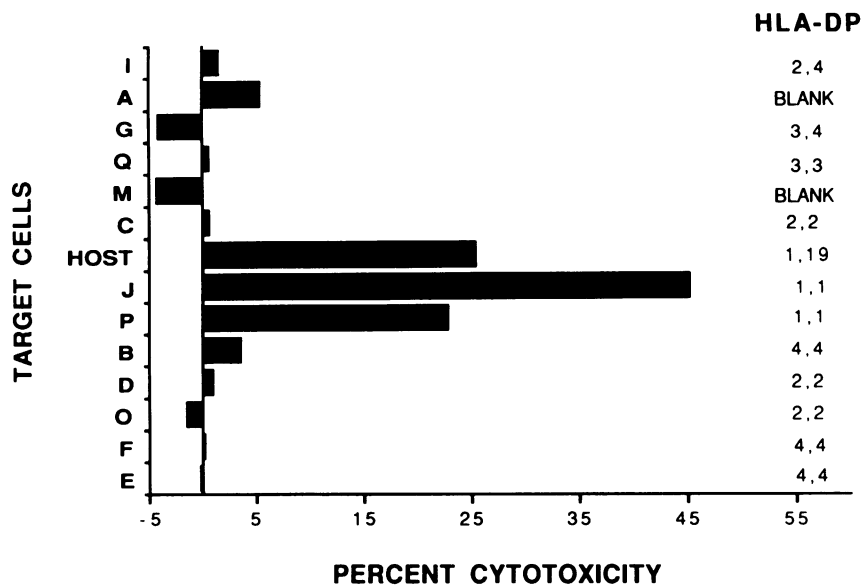


Figure 4. (Top) Specific lysis of HLA-DP1⁺ BLCL by SBD lymphocytes. For exhaustive typing of target cells, see Table I. (Bottom) Triggering of SBD T cell line proliferative activity by HLA-DP1⁺ BLCL. Assays were performed as for Fig. 2. For exhaustive HLA typing of target cells, see Table I.

bulk culture, as demonstrated by lysis of HLA-B27 BLCL HOM2 which was specifically inhibited by anti-HLA-B27 mAb HB157 (Fig. 8, top). Although CD8⁺ cells were overgrown by CD4⁺ in the bulk culture as in the case of patient 1, early cloning allowed us to obtain a CD8⁺ clone which specifically recognized the HLA-B27⁺ BLCL Q (WT24) and T (BTB) (Fig. 8, bottom). Because HOM2 was of female and WT24 and BTB of male origin, these data indicate as in the previous case that the CD8⁺ cells were not restricted to HLA-B27 of male origin.

Among the CD4⁺ clones characterized so far, two clones (CD4-1 and CD4-2) strongly proliferated when cocultured with irradiated BLCL R (BM14). This proliferation was blocked by mAbs 2D6 (anti-DP/DR), and B7.21 (anti-DP) but not by mAbs Leu-10 or 1A3 (anti-DQ) or GSP41 (anti-DR) (Fig. 9, top). When tested on panel BLCL, these two clones proved specific for BLCL G (DP301/401), R (DP401), S (DP401), but not for D (DP202), A (DP402), or P (DP101) (Fig. 9, bottom). Taken together, these results indicate that these CD4⁺ clones recognized HLA-DP401.

Discussion

Our data demonstrate the presence of T cells with defined anti-host specificity at a GVHD lesion site and indicate for the first time the direct involvement of DP antigens as a potential target for acute GVHD. More generally, they demonstrate the possibility of obtaining specific T cells from aGVHD lesions after nonspecific in vitro expansion of SBD lymphocytes, a technique which avoids the bias of in vitro sensitization. Because these cells were isolated from a lesion rather than from the periphery and were never restimulated in vitro with host cells before specificity assays, their specific anti-host reactivity probably reflects in vivo preselection. This is supported by the fact that we obtained six cell lines from 10 biopsies studied in different clinical situations. In the four cases in which the culture was negative, GVHD diagnosis was not confirmed. In the two cases presented, T cells started growing out of the biopsy within 3 d after initiation of the culture. These results are comparable to those obtained by Kasten-Sportes et al. (22), who also obtained T cell lines only when GVHD was confirmed. Recently,

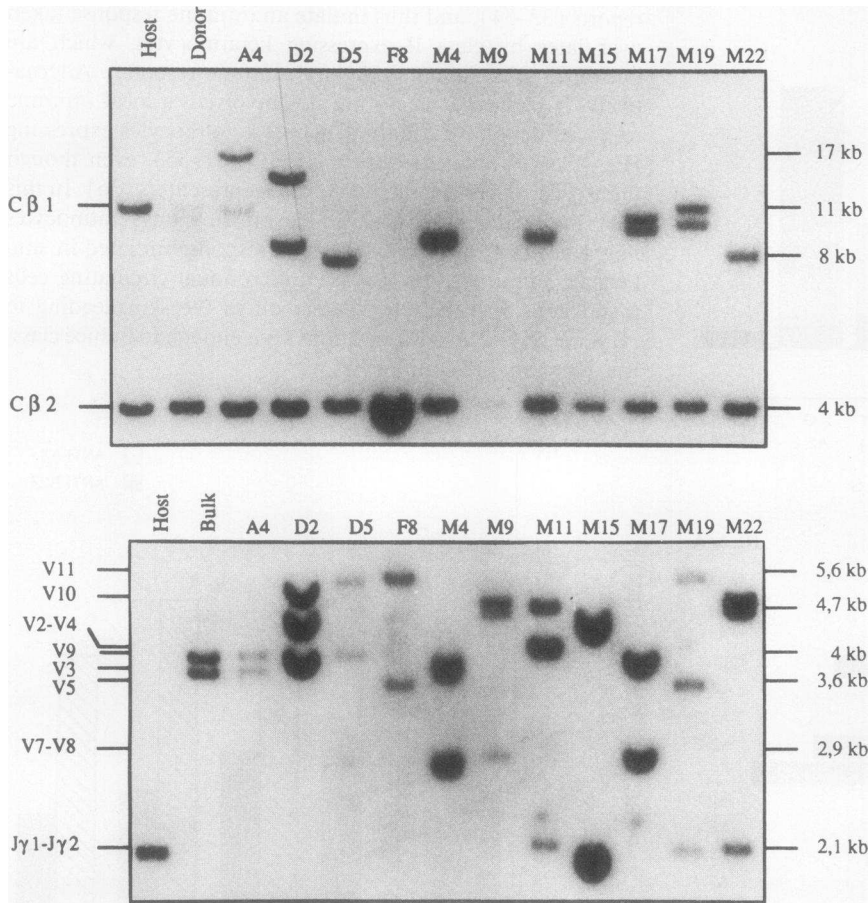


Figure 5. (Top) Southern blot analysis of TCR diversity among different clones derived from the SBD T cell line. Rearrangement of T-cell rearranging β genes in the different clones: DNA was digested with Eco RI and hybridized with pC β , a C β probe. Molecular size standards are shown at the right of the panel. (Bottom) Rearrangement of T-cell rearranging gamma genes in the different clones: DNA was digested with Hind III and hybridized with pH 60, a J γ probe.

in a study on renal allograft-infiltrating lymphocytes, Kirk et al. (27) strongly suggested that "... early evaluation of T-cell proliferation in vitro identifies activated T-cell infiltrates mediating acute cellular allograft rejection." Moreover, as in the present cases, these authors observed no correlation between the CD4/CD8 ratio of cultured cells and that deduced from immunohistochemical analysis. This lack of correlation be-

tween early in vitro culture and in vivo phenotype might reflect the microanatomical heterogeneity of the immune response. Although the CD8⁺ T cells were overgrown by CD4⁺ in long-term culture in both cases, early cloning allowed us to obtain two clones, each recognizing the class I mismatched allele between donor and recipient: HLA-A2 for patient 1 and HLA-B27 for patient 2. Taken together, our data and those cited

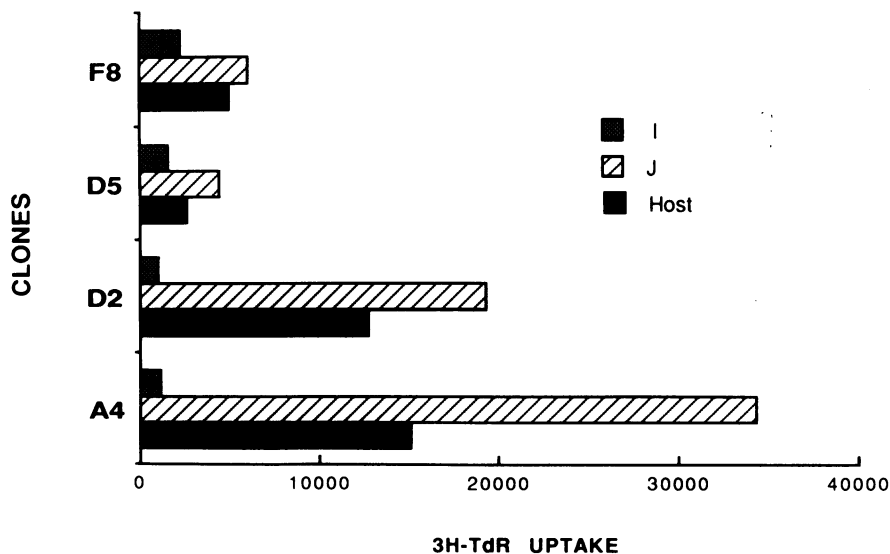


Figure 6. Proliferative activity of four different clones from the SBD T-cell line against host (DP101, 1901), I (DP202, 402), and J (DP101). Culture conditions were the same as for the cell line in Fig. 2.

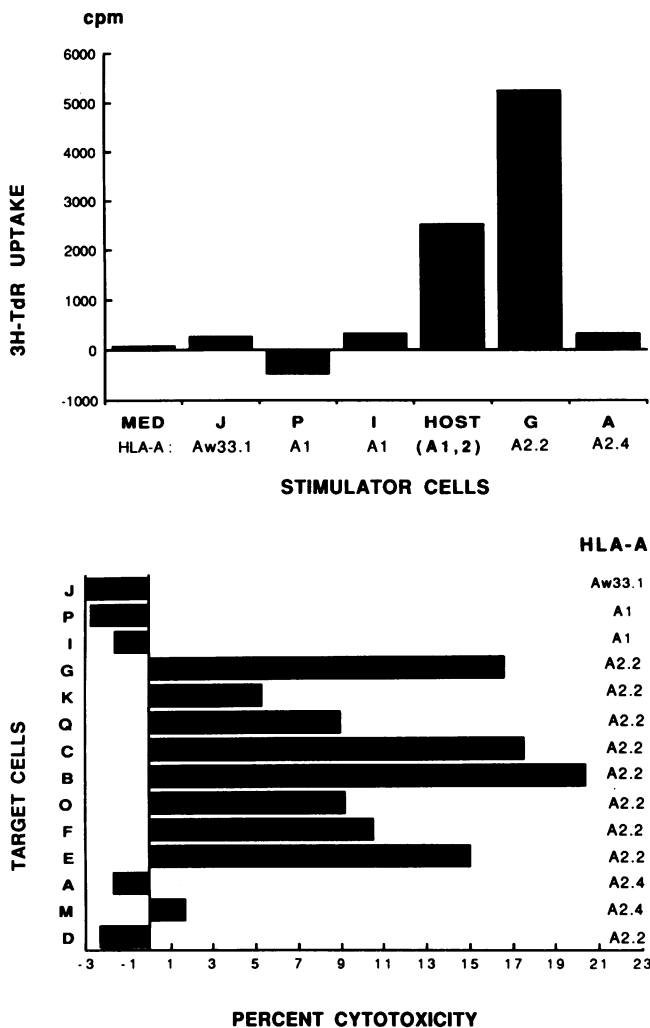


Figure 7. (Top) Clone M15 proliferative response. Assay performed in the presence of IL2 (150 BRMP U/ml). For exhaustive HLA typing, see Table 1. (Bottom) Specific recognition of HLA-A2.2 target cells by clone M15. All A2.2 target cells but one were recognized.

above strongly support the notion that cells able to proliferate in this system have a direct bearing on what is recognized in vivo.

The role of HLA-DP antigens in GVHD is controversial (28-30). Our data document the presence of specific CD4⁺ anti-DP T cells (against DP101 for patient 1 and DP401 for patient 2) at the site of a GVHD lesion 33 and 23 d after BMT, respectively. Together with recent evidence of a significant correlation between HLA-DP matching and a favorable outcome (30), this underscores the importance of extensive HLA typing before grafting. These results also raise 2 major questions regarding the nature of the Ag-presenting cells and the Ag itself:

First, are the antigen-presenting cells Langerhans cells or keratinocytes?

Langerhans cells are considered likely to induce strong immunization against MHC class II molecules. Although these cells are usually no longer detectable in the skin at the time of biopsy (31) and were not detected in the case of patient 1, they may still be present in small numbers, as suggested by previous

reports (32-34), and thus initiate an immune response taken over later by class II-expressing keratinocytes, which are known to stimulate a secondary immune response. Alternatively, if Langerhans cells are not involved, a local immune response may have occurred against keratinocytes expressing HLA class II antigens during GVHD (31-35), even though these cells are considered poor antigen presenters (36). In this case, induction of HLA class II expression on keratinocytes might not be related to an immune response initiated in situ. Instead, immunization against host residual circulating cells might have occurred elsewhere than in the skin, leading to cytokine release at concentrations high enough to induce class-II expression on keratinocytes.

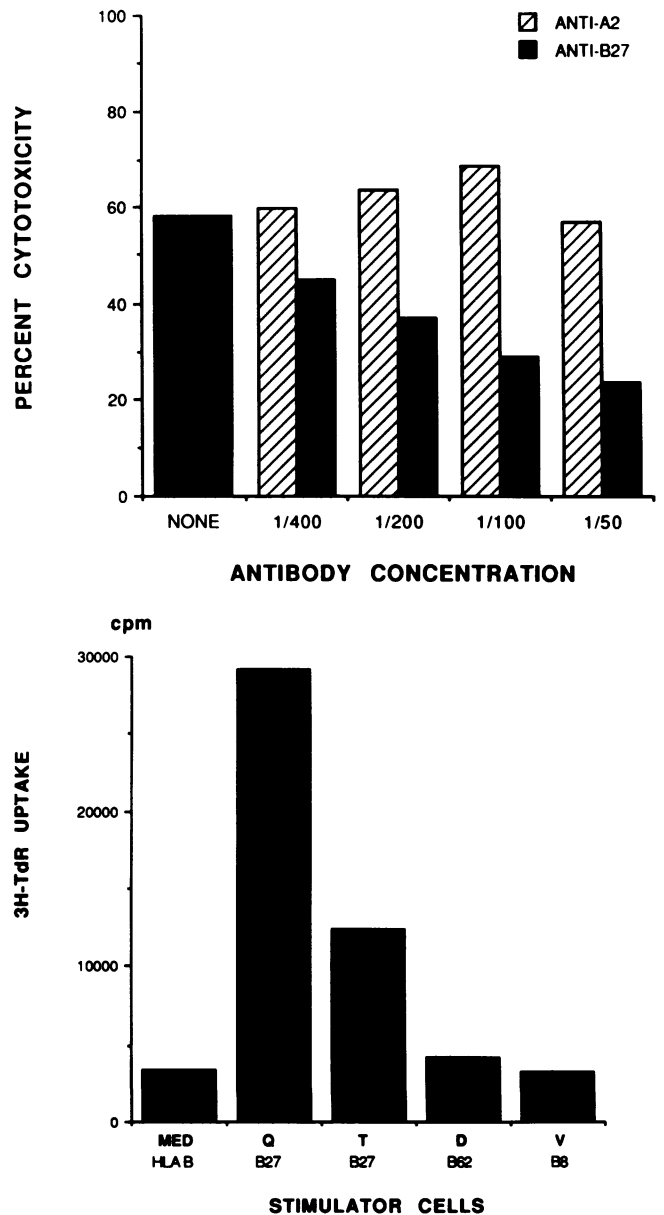


Figure 8. (Top) Cytotoxicity of the SBD T-cell line from patient 2 against the target BLCL HOM2 was specifically inhibited by mAb against HLA-B27. (Bottom) Specific recognition of HLA-B27 target cells by a CD8⁺ clone from patient 2. For exhaustive HLA typing, see Table 1.

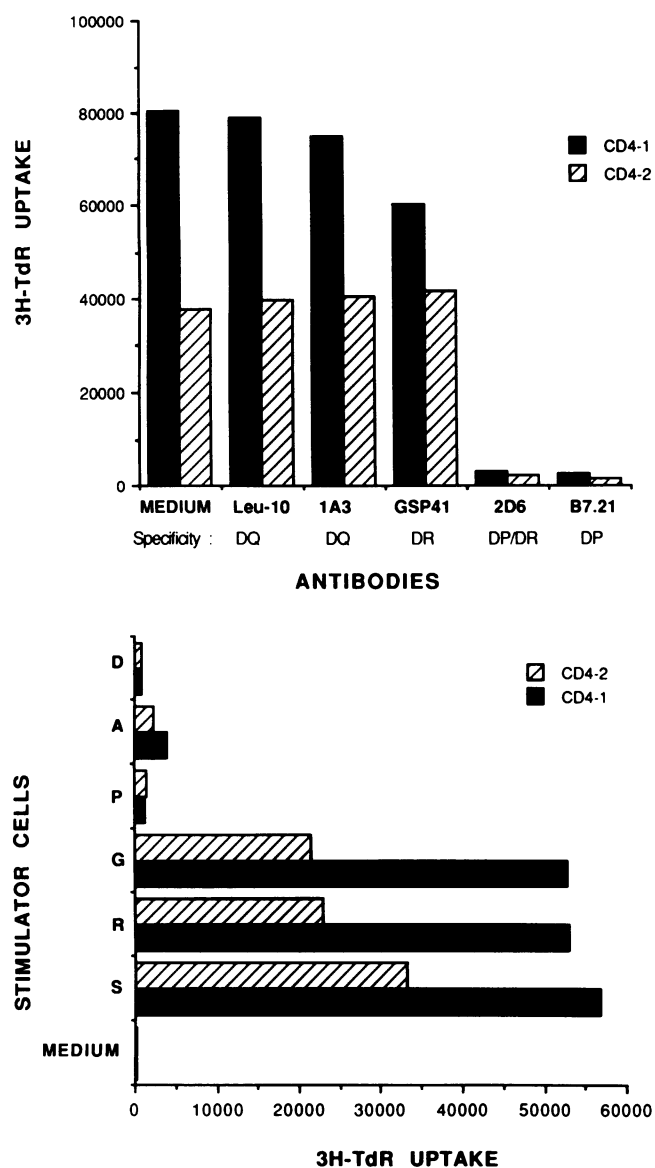


Figure 9. (Top) Recognition of target BLCL BM14 by clones CD4-1 and CD4-2 was blocked by mAbs against DP/DR (2D6) and DP (B7.21) but not by mAbs against DQ (1A3 and Leu-10) or DR (GSP41). Culture conditions were the same as for Fig. 2. Antibodies were used at 1/400 ascites dilution. (Bottom) Clones CD4-1 and CD4-2 specificity. Clones CD4-1 and CD4-2 were tested in a proliferative assay against the following panel of BLCL: D (HLA-DP202), A (HLA-DP402), P (HLA-DP101), G (HLA-DP301/401), R (HLA-DP401), and S (HLA-DP401). For exhaustive typing of target cells, see Table 1.

Secondly, what is responsible for the tissue specificity of the immune response?

HLA disparity is related to the appearance and intensity of the immune response during GVHD. However, tropism to epithelia is generally associated with GVHD. Consequently, the mechanism underlying this specificity should be at work in the different circumstances observed. As indicated by Parfray et al. (18) in a rat model, class-II antigen alone cannot account for the specificity of tissue injury because a high number of non-epithelial cells expressing HLA class-II in many tissues is not

affected by GVHD. These authors suggest that allogeneic T cells may have a higher affinity for class II antigens when these molecules are complexed to epithelial-specific antigens. The arguments in favor of this possibility are that T cells directed at non-physiologically presented autologous peptides are not deleted (37), that MHC molecules on different cell types may not be recognized in the same way by T cells (17, 38, 39), and that MHC class-II peptides involved in allorecognition may originate from exogenous proteins (40). However, this hypothesis does not fit very well with the data presented in this article because specificity studies were performed using BLCL cultured in the presence of 10% FCS. Nevertheless, the cytotoxic activity we observed was relatively low although specific (e.g., never > 35% at a 50:1 effector/target ratio through six independent determinations for clone M15). It is possible that the affinity of these T cells for their target would have been much better if the HLA molecules recognized were loaded with the "right" peptide. The T cells clones described here should allow us to clarify this point, which is essential to a clear understanding of the role of TCR target interaction in determining tissue specificity during GVHD. If TCR specificity is not the reason for GVHD tissue specificity, other putative candidates such as homing-associated adhesion molecules differentially expressed on various T cell subsets might be considered. In this view, mucosal and cutaneous lymphocyte-associated antigens (MLA or CLA antigens) (41, 42) would be the best candidates.

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