Correlation of Minor Histocompatibility Antigen-Specific Cytotoxic T Lymphocytes With Graft-Versus-Host Disease Status and Analyses of Tissue Distribution of Their Target Antigens

By Dietger Niederwieser, Alfred Grassegger, Josef Auböck, Manfred Harold, David Nachbaur, Agathe Rosenmayr, Anneliese Gächter, Walter Nussbaumer, Sabine Gaggl, Michael Ritter, and Christoph Huber

Peripheral blood mononuclear cells (PBMC) from 17 patients receiving HLA-identical sibling bone marrow grafts were stimulated with host pretransplant PBMC. Cytotoxic T-cell lines (TCL) with specificity for host pretransplant PBMC were obtained from 9 of these patients, all presenting with severe graft-versus-host disease (GVHD), but from none of the remaining cases lacking evidence of disease. Cytotoxic TCL were specific for host targets and failed to lyse donor cells. Monoclonal antibodies (MoAbs) blocking experiments and donor population screening analyses demonstrated that minor histocompatibility antigen (MiHA)-specific lysis of host targets was restricted by class I major histocompatibility complex (MHC) determinants. Whereas hematopoietic cells such as phytohemagglutinin (PHA) blasts or lymphoblastoid cell lines were susceptible to lysis by MiHA-specific TCL, keratinocytes (K) representing the natural targets of GVHD were quite resistant. Quantitative radioimmunometric measurements indicated very low constitutive expression of class I MHC antigens on K targets, which was readily increased by treatment with interferon-γ (IFN-γ). IFN-γ treatment at the same time rendered these cells susceptible to lysis by MiHA-specific TCL.

Host leukemic cells of 3 patients were recognized by MiHA-specific TCL in a chromium release assay and in one experiment host leukemic cells were effectively killed and their growth specifically inhibited in a leukemia colony assay by a clone. These data demonstrate that (1) host-specific cytotoxic TCL are detected exclusively in the PB of patients with acute GVHD grades II through IV after allogeneic matched bone marrow transplantation, and (2) their target antigens are simultaneously expressed on several host cell lines, including lymphoblastoid cell lines, PHA blasts, leukemic cells, and K. We also extend previous findings by showing that, besides the expression of the nominal MiHA, the density of the restricting class I MHC elements also crucially determines the extent of TCL lysis. Because of its capacity to enhance class I MHC antigen expression, IFN-γ represents a key cytokine for determining the susceptibility of MiHA targets for lysis by TCL and clones, and in one patient a MiHA-specific clone recognized host leukemic cells and also inhibited host leukemic cell growth in a colony inhibition assay.

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BONE MARROW transplantation (BMT) represents the therapy of choice for treatment of various malignant and nonmalignant diseases.1-7 Despite the use of HLA-identical sibling donors, graft-versus-host disease (GVHD) remains a frequent complication after BMT, affecting approximately half of all recipients.8-11 GVHD is mediated by donor lymphocytes recognizing so-called minor histocompatibility antigens (MiHA) on HLA-identical host cells.12-14 In contrast to the functionally and biochemically well characterized major histocompatibility complex (MHC) molecules,15,16 knowledge about the structure, distribution, and function of MiHA antigens is still rather limited.13,19 The only well-defined MiHA in mice is the maternally transmitted MiHA MTF and the nuclear myxovirus-resistance protein Mx.20,21 Molecules associated with the male-specific H-Y antigen were described as MiHA in humans.22,23 Recent experiments indicate that MiHA are peptides from endogeneous cell proteins that bind to MHC and are recognized by the antigen-binding T-cell receptors.13,22,24-27

This study was aimed at isolating host-specific T-cell lines (TCL) from the blood stream of patients who underwent MHC-identical allogeneic BMT, and to correlate their occurrence with clinical expression of GVHD. Also investigated were the tissue distribution of MiHA, the patterns of MHC restriction of host-reactive TCL lysis, and their interaction with GVHD targets of epithelial or leukemic origin.

PATIENTS AND METHODS

Patients. Seventeen (11 male and 6 female) patients undergoing BMT from their HLA-identical and MLC-negative sibling donors for treatment of hematologic malignancies (acute myelogenous leukemia [n = 4]; acute lymphoblastic leukemia [n = 2]; lymphoma [n = 4]; myelodysplastic syndrome [n = 1]; and chronic myelogenous leukemia [n = 4]) and severe aplastic anemia (n = 2) were investigated. Clinical characteristics of these patients are given in Table 1. Patients with hematologic malignancies were conditioned with cyclophosphamide (2 × 60 mg/kg/d) and total body irradiation (either as single dose 10 Gy or fractionated 2 × 2 Gy/d on 3 consecutive days), whereas patients with severe aplastic anemia (n = 2) were pretreated with cyclophosphamide 50 mg/kg/d for 4 days. One patient with lymphoma (unique patient number [UPN] 57) received Carmustine (BCNU), etoposide (VP-16), and cyclophosphamide without irradiation before BMT. Cytoxan was used as GVHD prophylaxis either alone (n = 14)28 or in combination with methotrexate (n = 3).29 Nine of the 17 patients developed GVHD grades II through IV and were subsequently treated with high-dose methylprednisolone (Table 1).

Isolation of peripheral blood mononuclear cells (PBMC). Heparinized PB was taken from donors before BM harvest, from patients...
before transplantation and during GVHD, or, at the latest, 2 months after BMT, PBMC were separated on Ficoll-Isoaque (Lymphoprep, Nyegard, Norway), washed three times, frozen, and stored in liquid nitrogen.

Epstein-Barr virus (EBV) transformation. Pretransplantation recipient PBMC were resuspended in tissue culture medium at concentrations of $2 \times 10^6$/mL and mixed with the same amount of a mycoplasma-free EBV producer line B 95-8 supernatant and 200 ng/mL cyclosporin (Sandimmun; Sandoz, Vienna, Austria). On day 6 and every third day thereafter, 50% of the culture medium was replaced by fresh medium. Cyclosporin was added to the culture until the third week.

Phytohemagglutinin (PHA) blasts. PBMC ($1 \times 10^9$/mL) from the host or donor were incubated with 1% PHA (Difco, Detroit, MI) in a humidified 5% CO$_2$/95% air atmosphere at 37°C for 72 hours. For further growth, cells were cultured in 20 U/mL recombinant IL-2 (Hoffmann-LaRoche) and 1% PHA-M (Difco) were added to the culture. After 6 days of culture, host-specific cytotoxic clones were selected and further cultured as described above. All clones and TCL were tested routinely for mycoplasma contamination (Gen-Probe; Gen-Probe Inc, San Diego, CA), and were negative.

Establishment of clones. On day 14, TCL were counted and diluted to 0.5 cells/well in a U-shaped 96-well plate (Falcon; Becton Dickinson, Oxnard, CA), 5 pg/mL transferrin (Calbiochem-Behring), 0.4 µL hydrocortisone (Calbiochem-Behring), and $8 \times 10^{-4}$ mol/L adenine (Boehringer Mannheim, Vienna, Austria). The cultures were incubated in 3:1 mixture of Dulbecco Vogt modification of Eagle’s medium in Ham’s F12 medium (Flow Laboratories, Irvine, UK) supplemented with 10% fetal calf serum (FCS, Flow), 5 µg/mL insulin (Sigma, Munich, Germany), $2 \times 10^{-3}$ mol/L triiodo-L-thyronine (Serva, Heidelberg, Germany), $10^{-6}$ mol/L choleratoxin (Calbiochem-Behring, La Jolla, CA), 0.4 µg/mL transferrin (Calbiochem-Behring), and $8 \times 10^{-4}$ mol/L adenosine (Boehringer Mannheim, Vienna, Austria). The cultures were incubated at 37°C in a 10% CO$_2$/90% air atmosphere and, after 2 days of culture, human recombinant epidermal growth factor (a generous gift of Dr H. Green, Harvard University, Boston, MA). Cultures were kept in a 3:1 mixture of Dulbecco Vogt modification of Eagle’s medium in Ham’s F12 medium (Flow Laboratories, Irvine, UK) supplemented with 10% fetal calf serum (FCS, Flow), 5 µg/mL insulin (Sigma, Munich, Germany), $2 \times 10^{-3}$ mol/L triiodo-L-thyronine (Serva, Heidelberg, Germany), $10^{-6}$ mol/L choleratoxin (Calbiochem-Behring, La Jolla, CA), 0.4 µg/mL transferrin (Calbiochem-Behring), and $8 \times 10^{-4}$ mol/L adenosine (Boehringer Mannheim, Vienna, Austria). The cultures were incubated at 37°C in a 10% CO$_2$/90% air atmosphere and, after 2 days of culture, human recombinant epidermal growth factor (a generous gift of Dr George Nascimento, Chiron Corp, Emeryville, CA) was added at a final concentration of 10 µg/mL. Medium was changed three times weekly until K were confluent. For further expansion, K were subcultivated as described above. K of these secondary cultures were used as targets after incubation with human recombinant interferon-γ (rIFN-γ; Genentech, South San Francisco, CA; specific activity, $2 \times 10^7$ IU/mg protein) or human rIFN-α2 (Boehringer Ingelheim, Ingelheim, Germany; specific activity, $3.2 \times 10^8$ IU/mg protein) for 72 hours with concentrations ranging from 1 to 1,000

### Table 1. Characteristics of BMT Recipients

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<tr>
<th>U/PN</th>
<th>Diagnosis</th>
<th>Recipient and Donor HLA-DR</th>
<th>Recipient/Donor MLR</th>
<th>Recipient/Donor Sex</th>
<th>GVHD Prophylaxis</th>
<th>Organ Grade</th>
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Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoid leukemia; SAA, severe aplastic anemia; MDS, myelodysplastic syndrome; MLR, mixed leucocyte reaction; CyA, cyclosporin A; Mtx, methotrexate; S, skin; L, liver; G, gut; MP, methylprednisolone.
U/mL. Cholera toxin was not included in culture media during incubation with IFN, as it prevents binding to its receptor. IFN-treated and untreated adherent K were recovered by treatment with 20 mL trypsin-EDTA for 30 minutes at 37°C and thereafter resuspended in RPMI medium supplemented with 15% heat-inactivated pool serum. The cells were washed, counted, adjusted to 1 x 10^6 cells/mL, and used as targets in cytotoxic assays.

Isolation of chronic myelogenous leukemia (CML) cells and testing in soft agar colony assay. Leukemic mononuclear blood cells were collected from three BMT recipients with CML and cryopreserved. Cryopreserved cells were thawed for 1 minute at 37°C in a water bath, washed three times, and resuspended in culture medium. CML cells from the recipients and, as controls, from unrelated patients were used as targets in a conventional 4-hour Cr release assay as described below and in a leukemia colony inhibition assay as previously described by Mackinnon et al. In this assay, target cells were preincubated in liquid culture without effector cells or with irradiated (5 Gy) TCL clones at an effector:target (E:T) ratio of 10:1 for 18 hours and subsequently cultured in triplicate for colony-forming units granulocyte-macrophage (CFU-GM) in an agar culture system as previously described. CFU-GM colonies were defined as granulocytic, monocytic, or mixed aggregates containing more than 50 cells. They were scored under an inverted microscope on day 14. The number of colonies cultured from 10^6 CML cells in the absence of the TCL was considered 100%.

Cell-mediated lympholysis (cml) assay. CML assays were performed as previously described. In short, 1 x 10^6 target cells (both donor and recipient K, PHA blasts, lymphoblastoid cell lines, recipient and third-party leukemia cells) were labeled with 100 μCi Na^214^O^3_ (NEN, Dreieich, Germany; specific activity, 300 to 500 Ci/g chromate) for 120 minutes at 37°C. On average, the following labeling intensities were achieved: for lymphoblastoid cell lines, 1.8 ± 1.1 cpm/cell; for PHA blasts, 1.1 ± 0.9 cpm/cell; for leukemic cells, 0.45 ± 0.1 cpm/cell; for K, 5.3 ± 2.8 cpm/cell; and, for K pretreated with IFN-γ, 6.65 ± 4.4 cpm/cell; for K pretreated with IFN-α, 4.2 ± 2.3 cpm/cell. For cytotoxic assays, 5 x 10^5 labeled target cells were mixed with the effector cells (TCL or clones) in U-shaped microtiter plates at various ratios ranging from 25:1 to 1:1. After 4 hours of incubation at 37°C in a humidified 5% CO₂/95% air atmosphere, 0.1 mL of the supernatant was removed with a semiautomatic harvesting device and counted with a gamma-scintillation counter (LKB). Background counts were determined using antimouse Ig F(ab')2 (Amersham, Buckinghamshire, UK; specific activity, 5 μCi/μg antibody protein) for 1 hour. The cells were washed three times and lysed, adding 100 μL of 2 N sodium hydroxide to each well. The resulting solution was absorbed with a cotton tip and analyzed in a gamma liquid scintillation counter (LKB). Background counts were determined using antimouse Ig F(ab')2 (Amersham) only.

Statistical evaluation. Student's t-test, χ² test, and Wilcoxon matched pairs signed rank test were used to compute statistical differences.

RESULTS

Host-specific cytotoxic TCL are exclusively established from patients with GVHD. PBMC isolated from 17 allogeneic BMT recipients after grafting were stimulated with irradiated pretransplant PBMC. Primary mixed leukocyte cultures (MLC) were then supplemented with natural IL-2 and restimulated with MiHA⁺ lymphoblastoid cells derived from the respective recipient's pretransplant PBMC. Under these conditions and from a total of 59 TCL grown, cytotoxic TCL with specificity for host cells were exclusively obtained from those 9 patients presenting with grades II through IV GVHD. No such cytotoxic TCL were established in the 8 patients with GVHD grades 0 through 1. These differences were statistically significant (Table 2, P = 0.001). TCL were tested for their capacity to lyse donor or host pretransplant PHA blasts. Lysis of all 9 TCL was restricted to host targets, as shown by resistance of the HLA-identical donor cells to lysis, and was therefore designated MiHA-specific (Table 2, P < .01). The possibility that cytotoxicity was directed against splits of MHC class I antigens was eliminated either by HLA typing of the parents or by biochemical HLA class I subtyping using an isoelectric focusing method.
Table 2. Functional Characteristics of TCL Grown From the PB of Patients With GVHD Grades 0-I and Grades II-IV

<table>
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<th>GVHD Grades II-IV</th>
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<td>9</td>
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<td>No. of TCL with specificity for host cells</td>
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<tr>
<td>No. of TCL without specificity for host cells</td>
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Specific lysis of host PHA blasts (%)

| E:T ratio 25:1 | 1 (0-5)         | 53 (36-93) | P < .0001* |
| E:T ratio 12:1 | 0 (0-2)         | 50 (24-80) | P < .01†   |
| E:T ratio 6:1  | 0 (0-2)         | 41 (16-71) | P < .01†   |
| E:T ratio 3:1  | 0 (0-3)         | 25 (0-54)  | P < .01†   |

Spontaneous release (cpm)

| E:T ratio 25:1 | 219 (77-777) | 288 (19-1,289) |
| E:T ratio 12:1 | 2,334 (625-3,906) | 2,734 (477-8,948) |
| E:T ratio 6:1  | 100 (0-0) | 100 (0-0) |

Maximum release (% spontaneous release)

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<th>Specific lysis of donor PHA blasts</th>
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<td>E:T ratio 6:1</td>
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<td>E:T ratio 3:1</td>
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Spontaneous release (cpm)

<table>
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<th>Maximum release (cpm)</th>
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Values are the median, with minimum and maximum in parentheses.

FACs analysis of cytotoxic TCL with specificity for host cells showed a predominant CD8 positivity (79.9%) and only a low percentage (16.9%) of CD4+ cells (Table 3, UPN 53). In contrast, TCL without specificity for host cells were CD4+ (91.8%) and CD8- (Table 3, UPN 58). All TCL expressed the CD3 marker and consistently lacked the CD16 (NK-cell associated) marker (Table 3).

The two TCL UPN 37 and 53 were further cloned, and clones displaying host specificity were expanded for further testing. Host-specific cytotoxic clones also showed a CD3+, CD8+, CD16+, CD4+ phenotype (Table 3, UPN 53 clone 7) and the noncytotoxic clones a CD3+, CD8-, CD16-, CD4+ phenotype (Table 3, UPN 53 clone 2).

Expression of MiHA on various hematopoietic and non-hematopoietic host targets. Cytotoxic TCL specific for MiHA were used to screen various hematopoietic and nonhematopoietic cells from the respective hosts for expression of MiHA. Lymphoblastoid cell lines, PHA blasts, leukemic cells, and K of host and of donor origin were used as targets. MiHA, as defined by specific lysis, were detected on both hematopoietic and nonhematopoietic cells, but the amount of target cell killing was variable (Fig 1). Host lymphoblastoid cell lines and PHA blasts were more susceptible to lysis than K and leukemic cells. Expression of MiHA on these targets...
was also confirmed by experiments with the two clones. An identical pattern with preferential lysis of host lymphoblastoid cells and PHA blasts was again observed (Fig 2).

Class I MHC represents the restricting element of MiHA-specific TCL responses and its expression crucially controls target cell lysis. Published evidence suggests that recognition of MiHA+ targets by TCL is restricted by class I MHC antigens. Our MoAb blocking experiments also confirmed these findings (Fig 3). Treatment with MoAb against common determinants of MHC class I but not class II antigens inhibited cytotoxicity. Blocking by MoAb against framework determinants of MHC class I antigens was more efficient than MoAb against polymorphic determinants of HLA-A or HLA-B antigens. Results were further supported in experiments using clones (Fig 4). As shown, cytotoxicity of the clones (from UPN 37) was blocked by anti-MHC class I and anti-HLA-A2 MoAb. In contrast, MoAbs against MHC class II were not inhibitory.

Because MiHA-specific TCL responses had been shown to be restricted by class I MHC, the possibility was considered that relative resistance of certain target cells to lysis might be caused by inappropriate expression of the restricting determinants. To test this, quantitative radioimmunometric measurements of class I or class II MHC antigen densities were performed on a panel of hematopoietic and nonhematopoietic targets. Results are shown in Table 4. Quantitative expression of MHC antigens clearly differed among the various cells analyzed, showing high MHC antigen expression on T blasts and lymphoblastoid cell lines and low or absent on leukemic cells and resting K (Table 4). Considering the enormous differences in size of K and PHA blasts or lymphoblastoid cell lines, these differences in MHC class I antigen expression were even more pronounced. After incubation of K with rIFN-γ using doses up to 1,000 U/mL MHC class I antigen, expression values increased in a dose-dependent manner. As discussed in the previous paragraph, there was a close correlation between the amount of MHC class I antigen expression on different targets and their susceptibility to lysis (Figs 1 and 2).

Further evidence for class I MHC antigens as restricting elements of MiHA recognition by TCL was obtained in studies using PHA blasts from a panel of 57 unrelated donors. Results are demonstrated in Fig 5; no specific lysis was seen with donors who failed to share at least one HLA-A or -B antigen with the TCL effector line. On the other hand, PHA blasts sharing HLA-A24 (15%), HLA-A31 (67%), HLA-B44 (55%), or HLA-B5 (38%) were all susceptible in a portion
of the test population. No evidence was obtained for restriction of the MiHA-specific TCL response in this panel for a single HLA specificity. This was different in patient UPN 37, in whom the haplotypes HLA-A3, B5 and HLA-A2, B7 were involved. Testing of PHA blasts of 19 unrelated donors with clones derived from this patient strongly suggest that this MiHA-specific TCL response is restricted by HLA-A2 (Fig 6).

By its capacity to enhance class I MHC antigen expression IFN-γ renders K targets susceptible to lysis by MiHA-specific TCL. As discussed in the previous paragraph, K representing the natural targets for GVHD exhibited a low constitutive expression of class I MHC antigens and also display relative resistance to lysis by MiHA-specific cytotoxic TCL. We tested whether preexposure of K targets to IFN-γ would render them susceptible to lysis by TCL. Results shown in Fig 7 indicate that IFN-γ, but not IFN-α, renders these cells susceptible to lysis by MiHA-specific TCL. Lysis of IFN-γ preincubated cells was again inhibited by anti-class I MHC MoAb (data not shown), demonstrating the crucial role of increased MHC class I antigen expression. To exclude that lysis of K exposed to IFN-γ might be due to a toxic effect on K, natural killing experiments were performed. No difference in susceptibility to lysis by NK cells between IFN-γ–treated or untreated K was found (Table 5).

MiHA are expressed on host leukemic cells. In 3 patients suffering from CML, cytotoxic TCL specific for MiHA were used to test the presence of these antigens on host leukemic cells. As shown in Fig 1, leukemic cells were quite resistant to the lytic attack of TCL.

This effect was studied in more detail in a chromium release assay and leukemia colony inhibition test, using clones from patient UPN 37. In the Cr release assay, leukemic target cells were recognized by clones (specific lysis, 40%; E:T, 25:1; see Fig 2). The presence of MiHA on host leukemic cells was also confirmed by the leukemia colony inhibition assay. Growth of the patient’s tumor cell colonies was drastically inhibited by the clone isolated during GVHD. This effect was host leukemia-specific, because growth of third-party, HLA-mismatched leukemia cells was not affected by the clone (Fig 8).

**DISCUSSION**

We demonstrate here that (1) host-specific cytotoxic T lymphocytes are detected exclusively in the PB of patients with acute GVHD after allogeneic BMT between HLA-identical siblings and (2) their target antigens are simultaneously expressed on several host cell lines, including lymphoblastoid cell lines, PHA blasts, leukemic cells, and K.

Our data, which indicate that successful establishment of MiHA-specific TCL is restricted to patients with extensive
GVHD, contradict previous reports by Van Els et al., but are supported by our previous experiments in which the frequency of MiHA-specific cytotoxic T cells (CTL-p) was assessed by limiting dilution analyses (LDA) subsequent to allogeneic BMT. We observed that MiHA-specific CTL-p frequencies were undetectable in recipients of HLA-identical BMT lacking clinical signs of extensive GVHD. However, high frequencies were detected in all 3 patients suffering from grades II through IV GVHD. LDA of MiHA-specific CTL-p were also performed in 8 of the cases studied here. Four of them neither developed extensive GVHD nor gave rise to MiHA-specific cytolytic TCL and also exhibited MiHA-specific CTL-p frequencies of less than 1/300,000. Of the other 4 patients, two developed grade II GVHD, gave rise to establishment of MiHA-specific weakly cytolytic TCL, but also exhibited MiHA-specific CTL-p frequencies below the detection limit. The last 2 patients presented with grade III GVHD, gave rise to highly cytolytic MiHA-specific TCL and clones, and displayed very high frequencies of MiHA-specific CTL-p of 1/13,833 and 1/7,933, respectively. In both these cases, CTL-p frequencies were below the detection limit before onset of GVHD. Taken together, our data support the view that extensive GVHD is accompanied by massive enlargement of the MiHA-specific CTL-p pool and that this favors the establishment of specific TCL or clones. The contradiction between the present results and those presented by Van Els et al. might be explained by different detection methods and immunosuppression used in the prophylaxis of GVHD. Furthermore, timing might play an important role in isolating TCL. In patient UPN 49, TCL were isolated exclusively during GVHD, and not 10 days after GVHD had been successfully treated with high-dose methylprednisolone.

We further investigated the presence of MiHA on different host tissues and the role of the density of the restricting class I MHC elements. The presence of MiHA on hematopoietic cells and K has already been reported by Goulmy and others., but not from HLA-mismatched third-party leukemia. We found that MiHA recognized by donor antihost TCL are simultaneously expressed on host lymphoblasts, on host K, and in one case on host leukemia cells and therefore not restricted to a single cell lineage. The presence of MiHA on cells of different tissues, however, did not result in an equal susceptibility of these cells to TCL. The amount of cell killing varied among target cells and paralleled the constitutive expression of the restricting class I MHC antigens.

We now expand previous information in mice by demonstrating that, at least in part, differences in the surface density of the restricting class I MHC determinants between hematopoietic and epithelial targets account for this unexpected and so-far unexplained relative resistance of epithelial cells. Data presented here with MiHA-specific class I MHC-restricted TCL thus confirm our previous findings of relative resistance of MHC-disparate K to lysis by MHC-specific TCL. In this case, too, pretreatment of K with IFN-γ enhanced their susceptibility to MHC-specific TCL, and their response was also inhibited by anti-class I MHC antibodies.

The view of the crucial role of class I MHC expression on GVHD target tissues and of IFN-γ regulating this key parameter is also supported by recent in vitro and in vivo find-
ings. IFN-γ and tumor necrosis factor-α (TNF-α) have been shown to be directly involved in GVH reactions using an in vitro human skin explant model. In vivo, endogenous IFN-γ and TNF-α levels are elevated before and during clinical manifestation of GVHD. Neutralizing antibodies to TNF-α or IFN-γ were shown to prevent or modify GVHD. Increased serum levels of both the nonpolymorphic and the polymorphic chain of HLA-A, -B indicating enhanced biosynthesis were demonstrated to proceed and accompany GVHD. Understanding the role of IFN-γ in the pathomechanisms of GVHD has several implications. First of all, it provides an explanation for the frequent association between infections and GVHD. Infection also provides a rationale for using cytokine modulation to prevent GVHD.

Leukemic cells have previously been shown to be susceptible to lysis mediated by MiHA-specific cytotoxic T lymphocytes. However, in our analysis we found that a clone isolated from a patient after BMT recognized leukemic cells, and lymphoblastoid cells from the host. Although our search for graft-versus-leukemia effects mediated by an MiHA-specific clone is so far limited to one case, the data presented clearly demonstrate that clones that lyse K only after pretreatment with IFN-γ effectively kill and inhibit growth of native host leukemic cells. This raises the hope that GVHD and antileukemic resistance can be differentiated on the basis of their different cytokine requirements. Obviously, much more data are required to assess the relevance of this observation. Such studies are now in progress at our institution.

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Correlation of minor histocompatibility antigen-specific cytotoxic T lymphocytes with graft-versus-host disease status and analyses of tissue distribution of their target antigens