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Recognition of a B Cell Leukemia-Associated Minor Histocompatibility Antigen by CTL

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CTL directed against minor histocompatibility Ags (mHag) play a major role in antileukemia reactivity after HLA-identical bone marrow transplantation. Some of these mHag are restricted to hemopoietic cells, others show a broad tissue expression. Therefore, antileukemia reactivity is often associated with graft-vs-host disease. Here, we report the identification of a B cell leukemia-associated mHag, HB-1, recognized by a CD8+ CTL clone derived from peripheral blood of an acute lymphoblastic B cell leukemia patient who has been treated by HLA-matched bone marrow transplantation. Interestingly, the CTL clone that recognizes HB-1 exhibits specific cytotoxicity toward leukemic as well as EBV-transformed B cells, but not against untransformed B cells. Moreover, the CTL clone does not lyse PHA-stimulated T cell blasts, monocytes, and fibroblasts, indicating that HB-1 is mainly expressed by transformed B cells. Further analysis reveals that HB-1 is restricted by HLA-B44 (both B*4402 and B*4403) and that 28% of HLA-B44-positive individuals express HB-1. These findings demonstrate that leukemia-associated mHag with a restricted tissue distribution, such as HB-1, elicit CTL reactivity in vivo. These Ags are of potential use in immunotherapy against leukemia because they generate antileukemia reactivity that is not associated with graft-vs-host disease. The Journal of Immunology, 1997, 158: 560–565.

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3 Abbreviations used in this paper: mHag, minor histocompatibility antigens; BMT, bone marrow transplantation; GVHD, graft-vs-host disease; GVL, graft-vs-leukemia; B-ALL, B-acute lymphoblastic leukemia; EBV-LCL, Epstein-Barr virus lymphoblastoid cell line; iCAM-1, intercellular adhesion molecule 1; IMDM, Iscove’s modified Dulbecco’s medium; dNTP, 2’-deoxynucleoside triphosphate.

Materials and Methods

mAb and immunofluorescence analysis

The following mAb were used for immunofluorescence analysis or for inhibition of cytotoxicity: TS2/18 (CD2), SPV-T3b (CD3), RI7-7 (CD4), WT82 (CD8), L15 (CD11a), F10.2 (CD54), TS2/9 (CD58), CR130.4 (anti-TCRBV651), OT145 (anti-TCRBV657), E17.5F3 (anti-TCRBV17), W6/32 (anti-HLA-class I), and QS13 (anti-HLA-DR/DP). Immunofluorescence was performed by the indirect method. FITC-conjugated goat F(ab’)2 anti-mouse IgG and IgM (Tago Immunologics, Camarillo, CA) was used for staining followed by analysis by an Epics XL flow cytometer (Coulter Electronics, Hialeah, FL).

CTL cultures

CD8+ T cells were isolated from PBL of patient MP (a 42-yr-old woman with a B-ALL) 9 mo after an HLA-identical BMT using anti-CD8 immunomagnetic beads (Dynal, Olso, Norway). A CTL line was established by stimulating CD8+ T cells (5 X 10^5/ml) with irradiated leukemic cells (10^7/ml) and autologous donor PBMC as feeder cells (2.5 X 10^6/ml) in IMDM (Life Technologies, Paisley, Scotland) plus 10% FCS. On day 7, cells were restimulated with irradiated leukemic cells from the patient (10^5/ml), and 100 U/ml IL-2 (Glaxo, Geneva, Switzerland) was added. From day 14 on, cultures were expanded and restimulated weekly with irradiated EBV transformed-lymphoblastoid cell lines (EBV-LCL) of the patient pre-BMT (10^6/ml), 100 U/ml IL-2, and 5 ng/ml IL-12 (Hoffmann-La Roche, Nutley, NJ).
**Target cells**

Leukemic cells were collected from B-ALL patients at diagnosis. Fibroblast cell cultures were generated from bone marrow obtained from patient MP pre-BMT. Fibroblasts and EBV-LCL were cultured in IMDM plus 10% FCS. Monocytes were isolated after adherence to plastic. T cell blasts were generated by stimulating PBMC with 4 μg/ml PMA in IMDM plus 10% human serum for three days. T cell blasts were washed and further cultured with 100 U/ml IL-2 for three days. B cells were obtained by positive selection using anti-CD19 immunomagnetic beads (DynaB). B cell blasts were generated by stimulating 10^6 CD19+ B cells with 5 × 10^4 CD32-transfected mouse fibroblastic L cells and 0.5 μg/ml CD40 mAb for 2 to 4 days. To increase susceptibility of B-ALL cells, fibroblasts, and CD40-stimulated B cells to specific CTL lysis, these cells were incubated with 10 ng/ml TNF-α (Boehringer Ingelheim, Alkmaar, The Netherlands) for 2 days.

**Chromium release assay**

Chromium release assays were performed as previously described (19). Fibroblast targets were labeled with 150 μCi ^51Cr for 18 h (20).

**IFN-γ release assay**

EBV-LCL and B cell blasts were tested for their ability to stimulate the production of IFN-γ by the CTL. Briefly, 10^6 CTL were cultured with 3 × 10^5 target cells in 200 μl IMDM plus 10% FCS and 25 U/ml IL-2. After 24 h, supernatant was collected and its IFN-γ content was determined by ELISA (CLB, Amsterdam, The Netherlands).

**HLA-B44 subtyping**

PCR cycles were amplified as described (21). HLA-B44 exon 3 DNA was amplified by PCR using 50 pmol B44EX3F primer (5'-TCTCCTCCGGGTATGACCAGG-3'), 50 pmol B44EX3R primer (5'-ACGGACTCCACGCCGGTCG-3'), 0.5 mM dNTPs and 2.5 U Taq polymerase (Life Technologies, Gaithersburg, MD) as previously described (22). PCR products were digested with PvuII to discriminate between HLA-B*4402 and B*4403.

**Cloning and sequencing of TCRB gene rearrangement**

Total RNA from 10^6 cells was extracted using the RNeasy method (Clontech Laboratories, Friendswood, TX) and reverse transcribed using an oligo(dT) primer and reverse transcriptase (Life Technologies, Gaithersburg, MD). TCRB cDNA was amplified by PCR using 250 pmol Cj8-N2 primer (5'-CACAGCGACCTCGGGTGGG-3'), 250 pmol V2-37 primer (5'-CCGGATCCTGTGTTCCAAGCCGGCC-3'), 0.5 mM dNTPs and 2.5 U Taq polymerase (Life Technologies, Gaithersburg, MD) as previously described (23). PCR products were cloned into pCRII vector by using the TA cloning kit (Invitrogen, San Diego, CA). Transformants were sequenced by the dideoxynucleotide chain termination method, and sequencing products were resolved on polyacrylamide gels.

**Results**

**Isolation of antileukemic CD8+ CTL**

To identify mHag expressed by leukemia cells, we isolated and expanded CTL from patient MP by stimulating CD8+ T cells, obtained after HLA-identical BMT, with irradiated B-ALL cells and used autologous donor PBMC as feeder cells. This CTL culture showed specific cytotoxicity against EBV-LCL of patient origin (51% specific lysis; E:T ratio 10:1), whereas EBV-LCL of the HLA-identical donor were not lysed. The CTL were of donor origin and expressed TCRαβ and CD8. Interestingly, TCR repertoire analysis of this CTL culture showed that 21 of 23 cloned TCRB cDNA expressed an uniqueBV6S1-DEAPEG-JB2S1 rearrangement (Table I). Eighty percent of the cells expressed TCRBV6S1 analyzed by flow cytometry (Table I).

Next, TCRBV6S1-expressing cells were sorted by flow cytometry. This CTL clone, MP1, efficiently lysed EBV-LCL of patient MP, whereas EBV-LCL of the HLA-identical donor BP were not killed (Fig. 1A). Lysis of K562 cells was not observed. Furthermore, we observed that B-ALL cells of patient MP preincubated with TNF-α were lysed, whereas untreated B-ALL cells were not killed (Fig. 1B). Lysis of B-ALL cells was efficiently inhibited by CD3, CD8, and HLA-class I mAb, whereas mAb directed against CD4 and HLA-class II were ineffective (Fig. 1C). These results demonstrate that CTL clone MP1 is directed against an HLA-class I-restricted mHag, designated HB-1.
Identification of the restriction element of HB-1

To determine the HLA molecule that presents HB-1 to CTL clone MP1, we tested EBV-LCL of relatives of patient MP. The results in Table II demonstrate that CTL clone MP1 recognizes HB-1 on EBV-LCL of three family members sharing expression of HLA-A33 and -B44 with the patient. Like EBV-LCL of the donor, EBV-LCL of one other HLA-A33, B44-positive family member does not express HB-1. These results demonstrate that HB-1 is recognized in association with HLA-A33 or -B44. To further define the HLA restriction molecule, we tested EBV-LCL of six sibling pairs unrelated to patient MP expressing HLA-A33 or -B44. EBV-LCL of one of these individuals, sharing only HLA-B44 with patient MP, were lysed by CTL clone MP1, thus demonstrating that HB-1 is presented by HLA-B44 (Table II). The observation that EBV-LCL of three out of ten randomly selected unrelated HLA-B44-positive individuals were also lysed confirms these data (Table II).

Expression of HB-1 by B-ALL cells

Since the HB-1-specific CTL clone MP1 was expanded by stimulation with B-ALL cells, we tested B-ALL cells of randomly selected HLA-B44-positive patients for recognition by CTL clone MP1. Leukemia cells of two out of eight B-ALL patients were HB-1 positive (Fig. 2). B-ALL cells of patient VR were only lysed upon TNF-α pretreatment with TNF-α, like B-ALL cells of patient MP. Interestingly, B-ALL cells of patient SC were recognized by HB-1-specific CTL without TNF-α pretreatment (Fig. 2). To determine whether B-ALL cells in general show low susceptibility to CTL-mediated lysis and whether this can be enhanced by TNF-α, we tested these cells for lysis by an anti-HLA-A2 CTL. TNF-α pre-incubation of B-ALL cells of patient MP and VR increased significantly the susceptibility to lysis by the HLA-A2 allospecific CTL line 1E2 (Fig. 2). In an attempt to explain the enhanced susceptibility of B-ALL cells to HB-1 specific and anti-HLA-A2 CTL lysis upon TNF-α treatment, we analyzed expression of MHC class I and adhesion molecules LFA-1, LFA-3, and ICAM-1 of B-ALL cells incubated with and without TNF-α. TNF-α clearly enhanced expression of ICAM-1 and LFA-3 of B-ALL cells (Table III). Lysis of B-ALL cells incubated with TNF-α was completely inhibited by a combination of anti-LFA-3 and anti-ICAM-1 mAb (Table IV). These data demonstrate that HB-1 is expressed by these B-ALL cells, but that significant expression of adhesion molecules is a prerequisite for lysis of B-ALL cells by CTL clone MP1.

Tissue specificity of HB-1

To determine whether HB-1 is expressed by all host cells or shows a restricted tissue expression, we tested lysis of fibroblasts and normal hemopoietic cells of patient MP, and three other HLA-B44, HB-1-positive individuals. Interestingly, PHA-stimulated T cell blasts, monocytes, and TNF-α treated fibroblasts were not lysed by CTL clone MP1, indicating that HB-1 is restricted to the B cell lineage (Fig. 3A). All cell types were efficiently killed by the
HB-1 is recognized in association with HLA-B44, which is a common HLA-B allele expressed by 23% of the Caucasian population (25). Among randomly selected HLA-B44-positive individuals we found that HB-1 is expressed by 3 of 10 EBV-LCL and by 2 of 8 B-ALL, resulting in a phenotype frequency of 28% (5/18). Since amino acid substitutions among HLA subtypes can affect the presentation of peptides to specific T cells, it is possible that subtype differences bias the phenotype frequency of HB-1. Five subtypes of HLA-B44 have been found, but the most frequently expressed subtypes are HLA-B*4402 and B*4403 (26, 27). HLA-B*4402 differs from HLA-B*4403 by a single amino acid substitution from Asp*4402 to Leu*4403 in position 156 of the α3 domain (28). Both HLA-B*4402 and -B*4403 were able to present HB-1 to CTL clone MP1 (Table II and Fig. 2). This is consistent with the finding that the peptide-binding motif of both subtypes is identical (29).

B-ALL cells of two out of three HLA-B44, HB-1-positive patients were only lysed by CTL clone MP1 after preincubation with TNF-α. This finding is in accordance with reports that lymphatic leukemia cells are less susceptible to lysis by CTL in vitro than myeloid leukemia cells and normal hematopoietic cells (14). We investigated whether the absence of several adhesion molecules by B-ALL cells might be the cause of low susceptibility to CTL lysis. We found that resistance to lysis correlated with low expression of ICAM-1 and LFA-3 by B-ALL cells. Susceptibility to CTL-mediated lysis of B-ALL cells was increased after incubation with TNF-α, which was clearly associated with an increase in ICAM-1 and LFA-3 expression.

Leukemic relapse after allogeneic BMT is a serious problem. Infusion of mHag-reactive donor T cells will always result in broad reactivity, inducing severe GVHD. Since HB-1 is expressed by B cells after malignant or EBV transformation, it may be an excellent Ag to develop immunotherapeutic protocols to eradicate
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FIGURE 3. Tissue-specific expression of HB-1. **A,** Cytotoxicity against EBV-LCL, PHA-stimulated T cell blasts, monocytes, and fibroblasts of HLA-B44, HB-1-positive individuals. The E:T cell ratio was 1:1. **B,** Production of IFN-γ by CTL clone MP1 stimulated with CD40 activated B cell blasts and EBV-LCL of HLA-B44, HB-1-positive individuals. B cells were stimulated with CD40 and 100 U/ml TNF-α for 2 days. One representative experiment of two is shown.

residual B cell leukemia cells in BMT patients without the development of severe GVHD. The low ICAM-1 and LFA-3 expression by some B-ALL cells raises the issue of tumor escape to HB-1 specific CTL. However, serum levels of the inflammatory cytokines TNF-α and IFN-γ are increased in BMT recipients during GVH reactions and viral infections (30, 31). TNF-α and IFN-γ can induce or up-regulate expression of MHC and adhesion molecules on residual leukemia cells in BMT recipients. In our view, it is therefore likely that B-ALL cells in BMT recipients will be susceptible to mHag-specific CTL.

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References


