The antileukemia effect of HLA-matched NK and NK-T cells in chronic myelogenous leukemia involves NKG2D–target-cell interactions

Giuseppe Sconocchia, Michelle Lau, Maurizio Provenzano, Katayoun Rezvani, Wachanan Wongsena, Hiroshi Fujiwara, Nancy Hensel, Jos Melenhorst, Jonming Li, Soldano Ferrone, and A. John Barrett

To study natural killer (NK) cell–mediated antileukemic activity in chronic myelogenous leukemia (CML), we investigated the ability of HLA-matched and mismatched CD56+ cells to inhibit granulocyte macrophage–colony-forming unit (CFU-GM) formation by leukemic CD34+ cells. In 14 HLA-identical donor-recipient pairs, donor CD56+ cells inhibited CML CFU-GM comparably to effectors from 14 HLA-mismatched unrelated individuals (mean inhibition 42% ± 9% vs 39.5% ± 7% at a 10:1 effector-to-target (E/T) ratio), suggesting that killer inhibitory receptor (KIR) incompatibility was not essential for an antileukemic effect. Both CD56+CD3− (natural killer [NK]) and CD56+CD3+ (NK-T) cells inhibited CFU-GM growth of CML but not normal CD34+ cells. A mechanism for this leukemia-specific cytotoxicity was suggested by the abnormal overexpression of major histocompatibility class I chain–related gene A or gene B (MICA/B) on CML CD34 cells and their ability to bind the NK activation ligand NKG2D.

Patients and methods

Patients and healthy subjects

Lymphocytes and CD34 cells were obtained from patients with CML, their healthy stem-cell donors, and other family members. Subjects gave written informed consent for donating cell samples by venepuncture or leukapheresis. Donors gave permission for a proportion of their peripheral-blood stem-cell (PBSC) transplant product to be used for research. All samples were collected under institutional review board–approved National Heart Lung and Blood Institute (NHLBI) clinical research protocols. Collected cells were divided into aliquots and cryopreserved in liquid nitrogen in 10% dimethyl sulfoxide (DMSO) until use.

Antibodies and reagents

Fluorescein isothiocyanate (FITC)–conjugated anti-CD56, anti-FcyRIII (CD16), anti-CD3, anti-KIR2DL1, anti-KIR3DL1, anti-CD94, anti–HLA class I antigen, phycoerythrin (PE)–conjugated anti-CD56, anti-CD16 and anti–HLA-DR, phycoerythrin-Cy5 (PE-Cy5)–conjugated anti–CD57, anticytotoxic killer inhibitory receptors (KIR) were purchased from Becton Dickinson, San Jose, Calif. FITC–conjugated anticytotoxic killer inhibitory receptor (KIR) incompatibility was not essential for an antileukemic effect. Both CD56+CD3− (natural killer [NK]) and CD56+CD3+ (NK-T) cells inhibited CFU-GM growth of CML but not normal CD34+ cells. A mechanism for this leukemia-specific cytotoxicity was suggested by the abnormal overexpression of major histocompatibility class I chain–related gene A or gene B (MICA/B) on CML CD34 cells and their ability to bind the NK activation ligand NKG2D. However, in vivo, CML cells may avoid NK-cell–mediated immune destruction by immune escape, shedding MICA into the plasma, thereby down-regulating NKG2D on CML CD56+ cells. (Blood. 2005;106:3666-3672)
mAb, matching isotype mouse mAb, Z-VAD-FMK, and Z-FA-FMK were purchased from BD Bioscience (San Jose, CA). PE-conjugated anti-CD34, anti-KIR2DL1, anti-KIR2DL2, and anti-NKG2A monoclonal antibodies (mAbs) were purchased from Beckman Coulter (Miami, FL). Magnetic bead–conjugated anti-CD34 and anti-CD54 mAbs and NK isolation kit “MiniMacs” magnet were purchased from Miltenyi Biotech (Auburn, CA). Allophycocyanin (APC)–conjugated anti-FAS (CD95) and PE-conjugated anti-FAS ligand (CD95L) mAbs were purchased from Caltag (Burlingame, CA). PE-conjugated and anti–human NKG2D mAb and a human recombinant NKG2D/Fe chimera were purchased from R&D Systems (Minneapolis, MN). Purified rabbit anti-MICA/B antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Interleukin-15 (IL-15), FLT3 ligand (FLT3-L), granulocyte macrophage–colony-stimulating factor (GM-CSF), and stem-cell factor (SCF) were purchased from Peprotech (Rocky Hill, NJ). Semisolid medium (MethoCult with or without recombinant cytokines) was purchased from Stem Cell Technologies (Vancouver, BC, Canada). Calcein AM was purchased from Molecular Probes (Eugene, OR).

**Cell isolation, activation, and expansion**

Isolation of total CD56⁺ cells (NK-T and NK). Cryopreserved peripheral-blood mononuclear-cell (PBMCs) collections were thawed, mixed with anti-CD56 mAb magnetic beads, and passed through a magnetic column (Miltenyi Biotech, Auburn, CA). CD56⁺ cells retained in the column were then eluted.

Isolation of NK cells. Peripheral-blood NK cells were negatively selected from PBMCs by magnetic sorting using the MiniMACS NK isolation kit (Miltenyi Biotech). Positively and negatively selected CD56⁺ cells or NK cells were stimulated in vitro in RPMI 1640 medium supplemented with 10% fetal calf serum and 2 mM glutamine, hereafter referred to as complete medium. In experiments to measure NKG2D expression, cells were cultured in serum-free medium (X-VIVO 10; Stem Cell Technologies) supplemented with GM-CSF, G-CSF, and SCF. Colonies (groups of 50 or more cells) were counted after 14 days.

Colony-inhibition assay

CD34⁺ cells in complete medium were plated in duplicate in the presence or absence of effector cells at an effector-to-target (E/T) ratio of 10:1 in 96-well U-bottom plates. After 5 hours of incubation at 37°C, in 5% CO₂, cells were transferred to 6-well plates containing methylcellulose (Methyl; Stem Cell Technologies) supplemented with GM-CSF, G-CSF, and SCF. Colonies (groups of 50 or more cells) were counted after 14 days. Results of duplicates target/effector combination were expressed as mean plus or minus standard deviation (SD).

Cytotoxicity assay

Replicates of 20 µL effector-cell suspension were serially diluted and incubated in 60-well (40 µL) plates (NalgeNunc International, Rochester, NY) for 30 minutes at room temperature. At the same time, 1 × 10⁶ to

**Table 1. Characteristics of study patients with CML**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Time since diagnosis, mo</th>
<th>WBCs x 10⁹/L</th>
<th>Diagnosis</th>
<th>Age/sex</th>
<th>HLA-Cw (Bw4⁺)</th>
<th>HLA-C group</th>
<th>Study performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>38</td>
<td>CML-AP</td>
<td>31/F</td>
<td>04/07 (−)</td>
<td>2/1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>33.5</td>
<td>CML-CP</td>
<td>23/F</td>
<td>05/07 (−)</td>
<td>2/1</td>
<td>1,2</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>12</td>
<td>CML-CP</td>
<td>29/M</td>
<td>04/05 (−)</td>
<td>2/2</td>
<td>1,2,3</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>3.5</td>
<td>CML-CP</td>
<td>32/M</td>
<td>03/07 (−)</td>
<td>1/1</td>
<td>1,3,5</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>23</td>
<td>CML-CP</td>
<td>26/M</td>
<td>04/15 (−)</td>
<td>2/2</td>
<td>2,3,5</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>112</td>
<td>CML-CP</td>
<td>23/M</td>
<td>07/16 (−)</td>
<td>1/NA</td>
<td>1,2</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>3</td>
<td>CML-CP</td>
<td>31/M</td>
<td>07/17 (−)</td>
<td>1/2</td>
<td>1,2</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>9.0</td>
<td>CML-CP</td>
<td>19/M</td>
<td>04/04 (−)</td>
<td>2/2</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>23</td>
<td>CML-CP</td>
<td>39/M</td>
<td>06/07 (−)</td>
<td>2/1</td>
<td>1,2</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>14</td>
<td>CML-CP</td>
<td>30/F</td>
<td>07/ND (−)</td>
<td>1</td>
<td>1,2</td>
</tr>
<tr>
<td>11</td>
<td>22</td>
<td>38</td>
<td>CML-CP</td>
<td>20/M</td>
<td>04/05 (−)</td>
<td>2/2</td>
<td>2,5</td>
</tr>
<tr>
<td>12</td>
<td>18</td>
<td>5.7</td>
<td>CML-CP</td>
<td>19/F</td>
<td>04/05 (−)</td>
<td>2/2</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>5</td>
<td>2.4</td>
<td>CML-CP</td>
<td>28/F</td>
<td>03/08 (−)</td>
<td>1/1</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>18</td>
<td>26</td>
<td>CML-PH</td>
<td>18/M</td>
<td>02/12 (−)</td>
<td>2/1</td>
<td>1,2</td>
</tr>
<tr>
<td>15</td>
<td>9.5</td>
<td>6.8</td>
<td>CML-CP</td>
<td>18/F</td>
<td>04/07 (−)</td>
<td>1/2</td>
<td>1,5</td>
</tr>
<tr>
<td>16</td>
<td>54</td>
<td>41.2</td>
<td>CML-AP</td>
<td>44/F</td>
<td>ND</td>
<td>ND</td>
<td>4</td>
</tr>
<tr>
<td>17</td>
<td>6</td>
<td>5.6</td>
<td>CML-CP</td>
<td>28/M</td>
<td>ND</td>
<td>ND</td>
<td>4</td>
</tr>
<tr>
<td>18</td>
<td>36</td>
<td>3.9</td>
<td>CML-CP</td>
<td>39/F</td>
<td>ND</td>
<td>ND</td>
<td>4</td>
</tr>
<tr>
<td>19</td>
<td>31</td>
<td>31.7</td>
<td>CML-CP</td>
<td>39/F</td>
<td>ND</td>
<td>ND</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>28</td>
<td>11.6</td>
<td>CML-PH</td>
<td>54/M</td>
<td>ND</td>
<td>ND</td>
<td>4</td>
</tr>
<tr>
<td>21</td>
<td>32</td>
<td>2.5</td>
<td>CML-CP</td>
<td>28/F</td>
<td>03/08 (−)</td>
<td>1/1</td>
<td>1,2</td>
</tr>
<tr>
<td>22</td>
<td>22</td>
<td>16.8</td>
<td>CML-CP</td>
<td>21/M</td>
<td>06/15 (−)</td>
<td>2/2</td>
<td>2</td>
</tr>
</tbody>
</table>

Due to the limited availability of the biologic material, the number of the assays performed for single patients varies.

CP indicates chronic phase; AP, accelerated phase; Ph, Philadelphia chromosome; NA, not available; ND, not done; 1, CD34 CML for colony inhibition assays with matched and mismatched effectors with or without acid stripping; 2, CD34 CML cells for MICA/B and NKG2D labeling; 3, CD34 CML cells for cytotoxicity tests; 4, serum assay for MICA/B; 5, CD34 CML cells for proliferation assay.
2 × 10^6 target cells were incubated in 1 mL complete medium supplemented with 10 μL Calcein-AM (Molecular Probes, Junction City, OR) for 30 minutes at 37°C, washed 4 times, and diluted to 1 × 10^5/mL. Subsequently, 10 μL target-cell suspension was added. Plates were then centrifuged and incubated at 37°C for 4 hours. A few minutes before scanning the plates, a fluorescent detector of 5 μL fluoro-quench was added to each well. The percent of lysis was calculated as follows: 1 – (mean test – mean blank) / (mean max – mean blank) × 100.

**Flow cytometry analysis of NKG2D ligand expression on CD34^+ cells**

Normal and CML CD34^+ cells were incubated on ice with rabbit or goat anti-MICA/B antibodies for 30 minutes. Goat or rabbit immunoglobulin G (IgG) were used as negative controls. After 2 washes, cell-surface–bound antibodies were detected using fluorescein-conjugated antibodies. The direct binding of NKG2D/Fc chimeric molecule was detected using a PE-conjugated anti-NKG2D mAb.

**Detection of MICA in serum**

The anti-MICA mAb, WW2G8 (IgG1) was generated in our laboratory (W.W., manuscript in preparation) using the methodology of Kohler and Milstein.26 The specificity of WW2G8 mAb was determined on bacteria (W.W., manuscript in preparation) using the methodology of Kohler and Milstein.26 The anti-MICA mAb, WW2G8 (IgG1) was generated in our laboratory (W.W., manuscript in preparation) using the methodology of Kohler and Milstein.26 The specificity of WW2G8 mAb was determined on bacteria (W.W., manuscript in preparation) using the methodology of Kohler and Milstein.26 The specificity of WW2G8 mAb was determined on bacteria (W.W., manuscript in preparation) using the methodology of Kohler and Milstein.26 The specificity of WW2G8 mAb was determined on bacteria (W.W., manuscript in preparation) using the methodology of Kohler and Milstein.26

To further investigate the antileukemia activity of HLA-matched CD34^+ cells, we first evaluated HLA-Cw alleles and the expression of KIR2DL1, KIR2DL2, and KIR2DL3 in peripheral-blood lymphocytes (PBLs) of the family members of patient 3. Subsequently, the patient's CD34^+ cells were cultured with HLA-matched and -mismatched CD34^+ cells from family members in a 4-hour cytotoxicity assay. The patient and the HLA-identical stem-cell donor expressed HLA-Cw4 and Cw5 haplotypes (group 2 NK specificity), whereas the patient's parents expressed Cw1 and Cw5 or Cw3 and Cw4 haplotypes (groups 1 and 2 NK specificity) allowing a comparison of KIR-matched and -mismatched effectors on the same target. Surprisingly, HLA-matched CD34^+ cells, obtained either directly from the donor or from the recipient following 100% donor engraftment, were more cytotoxic to the recipient CD34^+ CML cells than mismatched CD56^+ cells (Figure 1).

**CFU-GM inhibition.** The antileukemic activity of HLA-matched and -mismatched CD56^+ LAK cells against CD34^+ CML cells was then compared by the colony inhibition assay. In 14 paired comparisons of HLA-matched and -mismatched effectors against CML targets, there was no significant difference in the mean CFU-GM inhibition by HLA-matched (11 autologous, 3 allogeneic effectors), or HLA-mismatched CD56^+ cells (Table 3), indicating that HLA-matched and -mismatched CD56^+ cells had comparable antileukemic activity.

**Effect of HLA class I antigen expression on antileukemia effect of HLA-matched CD56^+ cells**

To evaluate the contribution of HLA class I antigens on CML CD34^+ cells to CD56^+-cell–mediated cytotoxicity, HLA class I antigen expression by CD34 cells was reduced by acid stripping.

Table 2. Phenotypic analysis of KIR expression on CD56^+ cells of the family shown in Figure 1

<table>
<thead>
<tr>
<th></th>
<th>HLA-Cw</th>
<th>HLA-C group</th>
<th>KIR2DL1</th>
<th>KIR2DL2</th>
<th>KIR3DL1</th>
<th>KIR2DL2/KIR2DL1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor</td>
<td>04/05</td>
<td>2/2</td>
<td>5</td>
<td>12</td>
<td>5</td>
<td>3.0/1</td>
</tr>
<tr>
<td>Recipient</td>
<td>04/05</td>
<td>2/2</td>
<td>4</td>
<td>11</td>
<td>7</td>
<td>2.8/1</td>
</tr>
<tr>
<td>Father</td>
<td>01/05</td>
<td>1/2</td>
<td>23</td>
<td>26</td>
<td>8</td>
<td>1.1/1</td>
</tr>
<tr>
<td>Mother</td>
<td>03/04</td>
<td>1/2</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>1.0/1</td>
</tr>
</tbody>
</table>

HLA-Cw4 and Cw5 bind KIR2DL1; HLA-Cw1 binds KIR2DL2; KIR3DL1 binds HLA-Bw4.
Most CML-cell samples lost surface HLA class I antigen expression within 2 minutes (without affecting CD34 expression; Figure 2). In 11 paired comparisons of colony inhibition by matched (n = 8) or mismatched (n = 3) CD56+ effectors against untreated or acid-stripped CML CD34+ cells, colony inhibition was significantly greater in the HLA-deficient targets (P = .01, Wilcoxon), indicating that target-cell cytotoxicity was in part blocked by KIR–HLA class I antigen interactions (Table 3). Since HLA class I antigens and KIRs are inherited separately, it is theoretically possible that CFU-GM inhibition could occur between HLA-identical effector-target pairs if individual NK clones express “orphan KIRs,” which do not bind to a cognate HLA class I ligand on the target. To test this possibility, the CFU-GM inhibitory effect of HLA-identical donors was compared in donor-recipient pairs where either homozygous for HLA class I antigen group 1 or 2 (n = 4), or heterozygous (expressing both group 1 and group 2 HLA class I antigens; n = 4). We found no difference in the inhibition of recipient CFU-GM between HLA-C group homozygous or heterozygous effectors (Table 3). These results eliminate an important role for autologous antileukemic effects through an orphan KIR mechanism.

**Antileukemia activity of CD56+ subsets**

Peripheral-blood CD56+ cells include a major subset of CD56−CD3− NK cells and a minor subset of CD56+CD3+ NK-T cells. To characterize the antileukemia activity of these subpopulations, CD56−CD3− and CD56+CD3+ cells were flow-sorted from the HLA-matched donors and leukemia patient 7. CML CFU-GMs were comparably inhibited by NK (CD56+, CD3−) and NK-T cells (CD56+, CD3+) from either the autologous source or from the HLA-identical donor (Figure 3A-B).

**HLA-matched CD56+ effectors have leukemia-restricted CD34 cytotoxicity**

To evaluate the specificity of the cytotoxic activity of HLA-matched CD56+ cells, we tested the effect of donor CD56+ cells in colony inhibition and direct cytotoxicity tests on the respective recipient CD34+ CML cells of patients 4 and 5 or on autologous CD34+ cells. The HLA-Cw alleles and KIR phenotype of HLA-matched CD56+ cells of patients 4 and 5 are shown in Table 4. Both HLA-matched donors showed cytotoxicity to patients’ CD34+ CML cells but not to normal autologous CD34+ cells, suggesting that only CML CD34+ cells expressed NK-activating molecules capable of overcoming KIR inhibition (Figure 3). Similarly, sorted and irradiated CD56+ cells, as well as the CD56−cell subpopulations KIR2DL1-KIR2DL2 and KIR2DL1-KIR2DL2, obtained in 2 separate experiments, inhibited the proliferation of CD34+ CML cells of patient 11, but had minimal or no inhibition against autologous (nonleukemic) CD34+ cells (data not shown).

**Expression of MICA/B on CD34+ CML cells**

To explore the possibility that CML cells express NK-activating ligands, we studied the expression of the NKG2D ligand MICA/B.
on CML CD34⁺ cells. MICA/B was expressed by CD34⁺ cells in 10 of 12 CML samples studied. Of the 4 MICA/B-positive patients tested, 2 showed strong binding of NKG2D; 1 bound weakly and 1 did not bind, possibly indicating functional polymorphism in MICA/B alleles. In contrast, normal CD34⁺ cells neither expressed MICA/B nor bound the NKG2D/Fc chimeric molecule, whereas the MICA⁺ HeLa-cell–positive control bound NKG2D/Fc (Figure 4).

NKG2D expression

To address the paradox that CML cells evidently persist in vivo despite the susceptibility of CML cells to NKG2D-mediated cytotoxicity in vitro, we examined NKG2D expression by NK cells of patients with CML. Three of 4 patients with CML (patients 10, 12, and 13) showed reduced expression of NKG2D by CD56⁺ CD3⁻ and also CD56⁺ CD3⁺ lymphocytes. In contrast, most healthy donor NK cells strongly expressed NKG2D. Reduced NKG2D expression appeared to be mediated by ligand-induced down-regulation, since expression by CML PBLs was restored after a 3-day culture in serum-free medium and IL-2 (Figure 5).

Shedding of MICA/B by CML cells and subsequent blocking of NKG2D on NK cells is another mechanism that might reduce in vivo autoreactivity against CML. We therefore assayed MICA in the serum of patients with CML and in healthy controls. Figure 6 shows that 2 of 5 patients with CML (patients 16 and 19) had detectable serum MICA and both had high leukocyte counts. In contrast, all of the normal sera tested had background levels of soluble MICA. This suggests that in vivo CML cells may escape NK-mediated cytotoxicity by shedding soluble MICA, thereby down-regulating NK cells by NKG2D blockade.

Mechanism of NKG2D-mediated cytotoxicity against CML CD34⁺ cells

Cytotoxicity by CD56⁺ cells was compared in 2 selected patients according to positivity for MICA/B expression (patient 2) or high affinity for NKG2D/Fc chimeric molecule (patient 14) and, using HLAA-matched and -mismatched (KIR incompatible) cells as effectors. To exclude a role for granule-independent cytotoxicity, experiments were carried out in the presence of Z-VAD-FMK, a general caspase inhibitor. CFU-GM colony formation in patients with CML exhibiting high NKG2D binding was clearly inhibited. Furthermore, this inhibition was partially reversed by an anti-NKG2D blocking antibody. These results suggest a role for NKG2D-ligand interaction in the regulation of HLA-matched CD56⁺-cell recognition of CML cells (Figure 7).

Discussion

Pioneering observations by Ruggeri and colleagues in the last few years have reawakened interest in the possibility of using NK-cell–based strategies to treat leukemia. These investigators have defined the KIR ligand disparities between recipient leukemia and donor NK cells required to elicit alloreactive cytotoxicity against leukemia cells. However, their results, derived from mismatched allogeneic stem cell transplantation studies, have to be reconciled with earlier observations that some leukemias are susceptible to autologous inhibition by NK cells, and that NK-cell susceptibility of leukemic cell lines is highly variable. These latter observations suggest that activating ligands on leukemia cells could counteract KIR-mediated inhibition and permit target-cell susceptibility in the absence of KIR incompatibility.

We chose to investigate the antileukemic potential of all CD56⁺-cell subsets in the context of CML, because previous studies showed that donor NK- and LAK-cell cytotoxicity can be readily generated against recipient CML in HLA-matched pairs, and NK-cell–mediated cytotoxicity against CML cells can be measured both by bulk cytotoxicity and by the CFU-GM inhibition assay. Furthermore, we previously described a correlation between NK/LAK activity after transplantation, and
the probability of sustained molecular remission in patients with CML.21 Thus, CML represents an informative model for studying mechanisms of NK cytotoxicity in autologous, HLA-matched, and -mismatched combinations of NK effectors with leukemia targets. In preliminary experiments, HLA-identical NK cells from healthy donors were found to inhibit CFU-GM growth in a dose-dependent fashion (Figure 1). Somewhat surprisingly, in a family study, the NK-cell–mediated cytotoxicity against CML from the HLA-matched donor was superior to that of the other HLA-mismatched, KIR ligand–mismatched family members. Furthermore, there was no significant difference between NK-cell–mediated colony inhibition by the HLA-identical donor and by other partially matched or completely HLA-mismatched NK-cell donors (Table 2), suggesting that the KIR inhibitory pathway was either inoperative or counteracted by pro cytotoxic effector-target interactions. Nevertheless, after removal of HLA class I antigen by acid stripping, we exposed a KIR ligand inhibitory effect, because both matched and mismatched CML progenitors were rendered more susceptible to NK-cell cytotoxicity after acid stripping (Figure 2; Table 2). Although it is possible that acid treatment may have rendered the targets more susceptible through mechanisms other than MHC class I removal, these findings appear to confirm the importance of KIR interactions in modulating antileukemic cytotoxicity but indicate that KIR inhibition in the HLA-matched setting is nevertheless overcome by pro cytotoxic NK-CML interactions.

In further experiments, we showed that both CD56+ NK and NK-T subsets had comparable cytotoxicity to HLA-matched targets, confirming observations by Mackinnon et al suggesting that LAK cells have antileukemia activity.4 However, cytotoxicity and inhibition of proliferation of CD34+ cells by HLA-identical NK cells was restricted to CML and was not seen against autologous normal CD34 cells, suggesting that CML cells abnormally express NK-cell–activating ligands. Since MICA/B is the natural ligand for the NK-cell–activating molecule NKG2D, we looked for MICA/B expression by CML CD34 cells. We found MICA/B expression on CML but not on normal CD34 cells, which correlated with the ability to bind soluble NKG2D, implicating an NKG2D-MICA/B interaction in CML target susceptibility. In one patient with a high level of MICA/B expression and high affinity for NKG2D chimeric molecules, we showed by blocking studies with anti-NKG2D that NKG2D was the dominant mediator of cytotoxicity. However, killing of CML cells by HLA-matched CD56+ cells was also observed in 2 patients with low or absent expression of MICA/B, suggesting cytotoxicity through FAS/FASL interaction28 or through other NK-cell–activating ligands such as ULBP.17

These findings raised the paradox that while CML progenitor cells are apparently highly susceptible to NK-cell regulation in vitro, autologous NK cells are self-evidently unable to prevent the leukemic process in the patient. Since NK cells in CML have been extensively studied and found to be functional,27,29 we considered the possibility that leukemia cells avoid autologous NK cells through immune escape. Consistent with this was the finding that patients with CML have detectable and potentially high levels of MICA/B in their serum, as has been described in other malignancies.30,31 MICA/B shed in the serum could block NKG2D on CD56+ cells, thus preventing NK-cell activation by the target. In support of this possibility was the finding of reduced NKG2D expression by NK and NK-T cells from some patients with CML, which increased after incubation in serum-free medium and IL-2 (in the absence of soluble MICA/B).

The clinical evidence for an antileukemic potential of NK cells is compelling, but the context of achieving these effects through mismatched stem-cell transplants is problematic because of significant treatment-related risks. Since our results indicate that NK cells kill CD34+ CML cells in the presence of intact KIR–MHC class I inhibitory pathways, it might be possible to enhance autologous or HLA-matched NK–malignant-cell interactions to achieve the same degree of efficacy as KIR ligand–mismatched interactions, but with greater clinical safety. To achieve this goal, methods to overcome immune escape through MICA/B shedding and NKG2D blocking would have to be developed. Alternatively, agents that up-regulate MICA/B on the leukemia cell might be helpful. MICA/B has a limited distribution in normal tissues. MICA/B is preferentially expressed in the gastrointestinal tract and thymocortical epithelia,15 but its expression is also induced by cell stress, viral infection, and malignant transformation.32,33 One strategy would therefore be to provoke stress in CML cells by hyperthermia or with interferons, retinoic acid, or chemotherapeutic agents. Earlier studies demonstrated that pretreatment of leukemia cells with actinomycin D or metabolic inhibitors increases leukemia-cell sensitivity to NK cell–mediated cytotoxicity,34 but whether this effect is mediated by the up-regulation of NK cell–activating ligands on leukemia cells is not known.

References

3. Hauch M, Gazzola MV, Small T, et al. Anti-leukemia potential of interleukin-2 activated natural killer...


The antileukemia effect of HLA-matched NK and NK-T cells in chronic myelogenous leukemia involves NKG2D–target-cell interactions

Giuseppe Sconocchia, Michelle Lau, Maurizio Provenzano, Katayoun Rezvani, Wachanan Wongsena, Hiroshi Fujiwara, Nancy Hensel, Jos Melenhorst, Jonming Li, Soldano Ferrone and A. John Barrett