A Soluble Factor Released by CD8⁺CD57⁺ Lymphocytes From Bone Marrow Transplanted Patients Inhibits Cell-Mediated Cytolysis

By Brigitte Autran, Véronique Leblond, Béhazide Sadat-Sowti, Evelyne Lefranc, Pierre Got, Laurent Sutton, Jacques-Louis Binet, and Patrice Debre

We report a new inhibitory activity of CD8⁺CD57⁺ peripheral blood lymphocytes from allo-bone marrow transplant patients. Positively selected CD8⁺CD57⁺ lymphocytes act as potent inhibitors of allospecific cytolytic T lymphocyte and lymphokine activated killer cell cytolytic activities. These suppressor cells are mature T cells expressing the CD2, 5, 7, CD3-TcRαβ complex, and lack natural killer cell markers as well as cytotoxic function. Their inhibitory activity on both cytoplasty processes and T-cell proliferation is mediated by a non–antigen-specific soluble factor released by CD8⁺CD57⁺ cells in culture supernatants. Preliminary characterization suggests that the CD8⁺CD57⁺ cells’ inhibitory activity is mediated by a low molecular weight, glycosylated factor as indicated by its less than 1S coefficient of sedimentation and its binding to concanavalin A lectin.

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MATERIALS AND METHODS

Patients. A total of 25 patients were studied from September 1989 to May 1990 after BMT from HLA-identical siblings for hematologic malignancies (24 cases) or severe aplastic anemia (one case). CMV infection was assessed on viral isolation in the blood or lungs. Blood bank donors (15) were tested as controls.

PBL. PBL were isolated by Ficoll-Hypaque density centrifugation (Pharmacia, Uppsala, Sweden) and either used immediately or cryopreserved.

Immunofluorescence (IF) analysis. A standard whole blood staining method was performed with the following monoclonal antibodies (MoAbs): anti-CD4-fluoro-isothiocyanate (FITC), anti-CD8-phycocerythrin (PE), and anti-CD57-FITC (Immunotech, Marseille, France). For three-color anti-IF analysis, isolated PBL were stained with biotin-conjugated anti-CD57 MoAb and various combinations of FITC- and PE-conjugated MoAbs: anti-CD2, 5, w29, 45RA (Coultronics, Margency, France); anti-CD3, 7, anti-TcRαβ, anti–HLA-DR (Becton Dickinson, Pont de Clay, France); and anti-CD8, 25, 71 (Immunotech). A PE-Texas-Red-streptavidin (Duochrom; Becton Dickinson) was used as second-step reagent.

Cell-sorting experiments. Fresh CD57⁺CD8⁺ doubly positive PBL were purified on a FACSTAR cytofluorometer (Becton Dickinson) in CD8⁺CD57⁺ cells (purity > 95%) versus CD8⁺CD57⁻ control cells. Avidin-conjugated magnetic beads (Immunotech) coupled with anti-CD57 biotin MoAb were incubated with fresh PBL for 1 hour at 4°C. Beads forming rosette-CD57⁺ cells were then sorted (enrichment > 75%) versus CD57⁻ cells containing less than 5% residual CD57⁺ cells.

Supernatant (SN) preparation. SN were prepared from either fluorescence-activated cell sorter (FACS) or immunomagnetic beads purified CD8⁺CD57⁺, or control CD57⁻ cells incubated at 5 × 10⁶ cells/mL in serum-free culture medium for 4 hours at 37°C without stimulation. After centrifugation, SN were filtered through 0.22-μm filters.

Cytotoxicity assays. CTL and LAK cells were generated from normal PBL. Alloreactive anti-HLA CTL were tested after a 5-day allo-mixed lymphocyte culture (allo-MLC) against allogeneic Epstein-Barr virus (EBV)-transformed B-cell lines. LAK cells were tested against Daudi target cells after a 3-day culture in RPMI medium supplemented with 1 mmol/L sodium pyruvate, 2 mmol/L L-glutamine, 10% fetal calf serum plus 100 U/mL recombinant interleukin-2 (rIL-2; Boehringer-Ingelheim, Grenoble, France), or phytohemagglutinin-P (PHA-P) (1 μg/mL; Wellcome, Paris, France). The 4-hour ⁵¹Cr release assays (CARA) were performed at various killer/target cell ratios. Lysis was expressed as percent of specific ⁵¹Cr release; spontaneous ⁵¹Cr release varied from 12% to 25% of total incorporated radioactivity. For inhibition assays of cytolytic activity, suppressor and control cells or SN were added to killer cells at various ratios at the onset of the CRA. Percentage of inhibition was calculated as follows:

\[
\% \text{Inhibition} = \frac{\text{Control Release (without suppressor cells)} - \text{Supernatant Release}}{\text{Control Release (without suppressor cells)}} \times 100
\]

Ten thousand cells were analyzed for fluorescence on a Facscan cytofluorometer (Becton Dickinson, San Jose, CA).

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Proliferation assays. Normal PBL were incubated 3 days with or without PHA-P 1 μg/mL, or in microtiter plates precoated with anti-CD3 MoAb (UCHT1; a gift from P. Beverley, London, UK) in RPMI-supplemented medium. Cells were pulsed with 1 μCi (3H) TdR (5 Ci/nmol; CEA, Orsay, France) for the last 18 hours. For inhibition assays of proliferation, dilutions of SN from purified CD8⁺CD57⁺ or control PBL were added at day 0 of the cultures. Percentage of inhibition was calculated as follows:

\[
\frac{(\text{cpm from responder cells alone} + \text{SN}) - (\text{cpm from responder cells alone} - \text{SN})}{(\text{cpm from responder cells alone} + \text{SN})}
\]

Glycerol gradient centrifugation and ConA-affinity chromatography. Concentrated SN (4×) from CD8⁺CD57⁺ or CD57⁻ cells were layered onto either: (1) linear (5% to 20%) glycerol gradients, centrifugation was performed at 50,000 rpm and 4°C for 18 hours, sedimentation coefficients were determined by using glucose oxidase (7, 9 sedimentation [S]) and peroxidase (3, 6S) as standards; or (2) a column of ConA-sepharose (Pharmacia, France) presaturated with bovine serum albumin (BSA; Sigma, St Louis, MO) and equilibrated at pH 7.6. After elimination of unbound material, specifically adsorbed compounds were eluted with 1 mol/L methyl-a-D-mannopyranoside (Sigma). Fractions (200 μL) were dialyzed before being tested for inhibition of LAK cell activity.

RESULTS

CD8⁺CD57⁺ PBL from BMT recipients inhibit cell-mediated cytolyis. PBL from 25 BMT recipients were prospectively analyzed for coexpression of the CD8 and CD57 antigens defining a group (1) of 13 patients with a major increase of the CD8⁺CD57⁺ subset (>15%, mean 31% ± 12%, ranges 15% to 59%) as compared with 12 patients in group II (<15%, mean 6% ± 5%, ranges 1% to 15%) and with normal controls (mean 7% ± 5%, ranges 1% to 12%) (P < .01). A CMV infection was significantly more frequent in group I (7 of 13) than in group II (1 of 12) (P < .05), but no differences could be detected concerning the delay from BMT, acute graft-versus-host disease (GVHD), or chronic GVHD (data not shown).

We then investigated whether CD8⁺CD57⁺ PBL isolated from allogeneic patients could inhibit HLA-specific cytolytic activities. Allospecific CTL were first generated from normal controls against appropriate allogeneic EBV-transformed B-cell lines. The addition of group I PBL to a constant number of CTL at the onset of CTA induced a dose-dependent inhibition of the allospecific cytolyis (Fig 1A). This inhibition of allogeneic CTL was not restricted by major histocompatibility complex (MHC) antigens and could be observed by using PBL from nine different HLA-mismatched group I patients (data not shown). Such a lack of MHC restriction prompted us to look for a similar suppression of non-antigen-specific cytolyis: rIL-2-driven LAK cell activity was similarly inhibited by PBL from a group I patient (Fig 1B), as were inhibited PHA-activated killer cells and natural killer (NK) cells (data not shown).

Table 1. Three-Color IF Analysis of CD8⁺CD57⁺ PBL From Allo-BMT Recipients

<table>
<thead>
<tr>
<th>Cell-Surface Antigens</th>
<th>Fraction of Positive CD8⁺CD57⁺ PBL [%]</th>
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<tbody>
<tr>
<td></td>
<td>UPN 1</td>
</tr>
<tr>
<td>CD2</td>
<td>95</td>
</tr>
<tr>
<td>CD7</td>
<td>100</td>
</tr>
<tr>
<td>CD3</td>
<td>94</td>
</tr>
<tr>
<td>TcRαβ</td>
<td>90</td>
</tr>
<tr>
<td>CD56</td>
<td>7</td>
</tr>
<tr>
<td>CD25</td>
<td>0.7</td>
</tr>
<tr>
<td>HLA-Dr</td>
<td>23</td>
</tr>
<tr>
<td>CDw29</td>
<td>100</td>
</tr>
<tr>
<td>CD45RA</td>
<td>56</td>
</tr>
</tbody>
</table>

PBL from two representative BMT recipients (UPN 1 and 2) were incubated with various combinations of MoAbs. IF analysis was performed on a Facscan system (Becton Dickinson). Results are given as percentages of positive cells within the cell population doubly labeled with the anti-CD8-FITC/PE and CD57-Duochrom MoAbs.

Abbreviation: UPN, unique patient number.
CD8'CD57' PBL display the CDw29 antigen and 56% to 58% display the CD45RA antigen, suggesting a possible coexpression of both antigens on CD8'CD57' cells.

CD8'CD57' PBL release a soluble nonspecific inhibitory factor. The nonspecific, short-term inhibitory activity of CD8'CD57' PBL suggested the release of a soluble inhibitor of cytolitic functions. Supernatants were then produced from purified CD8'CD57' PBL in a 4-hour culture in serum-free medium. Those SN mediated a dose-dependant inhibition of differentiated killer cell activity similar to that observed with the CD8'CD57' cells (Fig 2A). We then looked for a similar activity of CD8'CD57' SN on T-cell proliferation. Figure 2B shows that the addition of CD8'CD57' SN at day 0 of culture inhibits both PHA- and anti-CD3-induced proliferation of normal PBL as well as nonspecific or antigen-specific killer cell activity.

To define the target of such a soluble inhibitory activity, cytolytic effector and target cells were separately incubated in CD8'CD57' SN, then washed before CRA. As shown in Fig 2C, Daudi target cells remained sensitive to killer cells after preincubation, whereas LAK cells lost 66% of their cytolytic activity for the subsequent 4 hours. These SN were not lytic because we observed a 90% viability of LAK and target cells after a 4-hour incubation in CD8'CD57' SN (data not shown). Furthermore, we could demonstrate that prostaglandin E (PGE) was not responsible for the inhibitory effect described here because the CD8'CD57' effect was not abolished when CD8'CD57' PBL were treated by inhibitors of PG synthesis, such as indomethacin (data not shown). These data indicate that the soluble inhibitor present in CD8'CD57' supernatants is acting at the effector killer cell level.

Preliminary characterization of the soluble inhibitor released from CD8'CD57' PBL. The coefficient of sedimentation (CS) of the CD8'CD57' soluble factor was then defined by fractionation on the glycerol gradient of SN from both purified CD8'CD57' and CD57' cells; resulting fractions were tested for their inhibitory activity on LAK cell-mediated cytolyis indicating a CS less than 15 (Fig 3A) because the inhibitory activity of CD8'CD57' SN was present in fractions N 17 and 18, but not in fractions N 10 through 16 or 19 through 26 from CD8'CD57' SN or in fractions from CD57' SN. Finally, we tested the ability of the CD8'CD57' soluble inhibitory activity to bind to ConA lectin. We applied a fourfold concentrated CD8'CD57' SN to a ConA column previously saturated with BSA. As shown in Fig 3, the inhibitory activity totally disappeared in the unbound fractions, whereas it could be specifically eluted after addition of methyl-α-D-mannoside. Altogether, these data indicate that the CD8'CD57' lymphocyte inhibitory activity should be mediated by a glycosylated, low molecular weight soluble inhibitor.

DISCUSSION

This study was undertaken to investigate the ability of CD8'CD57' lymphocytes from allo-BMT recipients to inhibit cell-mediated cytolytic activities, which are supposed to play a major role in a context of transplantation.

Our results show that sorted CD8'CD57' PBL from allo-BMT patients not only lack spontaneous cytolytic activity against NK and LAK target cells, but also clearly inhibit both HLA-specific and non-antigen-specific killer cell activity. These data, together with our previous results obtained in HIV-infected patients, suggest that CD8'CD57' cells, lacking both HLA restriction and antigen specificity, are likely to interact with killer cells at a common step of the
cell-mediated cytolytic processes, independently of the antigen recognition events.

This suppression of cytolytic activity is mediated by a soluble inhibitor present in supernatants from sorted CD8+CD57+ cells. Furthermore, the CD8+CD57+ cell-mediated suppression of B- and T-cell proliferation already observed is also likely to be mediated by the same CD8+CD57+ inhibitory molecule, because CD8+CD57+ SN also inhibit the anti-CD3 or mitogen-induced T-cell proliferation. Our data indicate that CD8+CD57+ cells do not confer a state of resistance to lysis, but rather induce an impairment of the cytolytic effector cell function. Moreover, the viability of LAK and Daudi cells after incubation in CD8+CD57+ SN argues against a lytic mechanism responsible for this inhibition. Finally, the inhibitory activity of CD8+CD57+ SN can be detected in the less than 1S, ConA-binding fractions, suggesting the resistance of a mannosylated, low molecular weight molecule in this phenomenon and allowing further biochemical characterization. This soluble inhibitor differs from other known inhibitory or immunoregulatory molecules, such as transforming growth factor (TGF) or PGE2, as shown by its resistance to PGE2 synthesis inhibition or to TGFβ neutralization. To our knowledge, these original data constitute the first report on the mechanism of action of CD8+CD57+ lymphocytes isolated in BMT patients and add to the complexity of functions mediated by such T cells.

Interestingly, CD8+CD57+ lymphocytes display the CD3-TcRαβ complex, raising the question of the functionality of this complex, as previously suggested by the ability of CD8+CD57+ cells to generate in vitro an LAK cell function on a CD3 stimulation. Whether this killer function does occur in vivo is still unknown, but it should be emphasized that it is not detectable in vitro in nonstimulated CD8+CD57+ PBL from BMT recipients. Of note is the presence of the CDw29 antigen and a possible coexpression of the CD45RA antigen, suggesting that CD8+CD57+ cells might have been primed in vivo. Finally, the CD8+CD57+ PBL increase is usually associated with clinical conditions characterized by high antigenic stimulation, such as either persistent CMV, EBV, or HIV infections, or transplants. Contrasting with several reports, we could not see a relationship between the increase of such suppressor cells and GVHD, but we could observe a significant association with CMV infection, as previously mentioned.

Whatever their induction mechanism, the in vivo relevance of the inhibitory activity mediated by CD8+CD57+ cells remains to be determined. Further studies of the inhibitor released by CD8+CD57+ cells, currently in progress in our laboratory, should help us to better define the inhibition of lymphocytic functions described here and the CD8+CD57+ cells’ implication in an in vivo control of BMT recipients’ immune status.

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REFERENCES


7. Clement LT, Grossi E, Garlund GL: Morphology and pheno-
typic features of the subpopulations of Leu2+ cells that suppress B cell differentiation. J Immunol 133:2460, 1984


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