A LYMPHOKINE THAT ACTIVATES THE CYTOLYTIC PROGRAM OF BOTH CYTOTOXIC T LYMPHOCYTE AND NATURAL KILLER CLONES

BY CLAUDIO MILANESE,* ROBERT F. SILICIANO,* REINHOLD E. SCHMIDT, JEROME RITZ, NEIL E. RICHARDSON,* AND ELLIS L. REINHERZ

From the *Laboratory of Immunobiology and Division of Tumor Immunology, Dana-Farber Cancer Institute and the Departments of Medicine and Pathology, Harvard Medical School, Boston, Massachusetts 02115

Mature T lymphocytes can be activated through either the T3-Ti molecular complex (1) or the T11 structure (2) to undergo proliferation and mediate various regulatory functions. While the natural ligand for the former is clearly antigen and MHC, the natural ligand of the T11 structure has only recently been identified (3). In this regard, it has been shown that a novel lymphokine with an apparent molecular mass of 10-12 kD is secreted from Th clones within hours after crosslinking of their antigen/MHC receptors. This lymphokine, herein termed T cell activating factor (TCAF),1 stimulates resting T lymphocytes via binding to surface components of the alternative T11 pathway. The activation process is not dependent on either antigen specificities of recruited populations or the presence of macrophages and IL-1. It thus appears that TCAF is a mediator involved in amplifying the T cell immune response through recruitment of other cellular components of the lymphoid system.

mAbs directed against spatially distinct epitopes of the T11 molecule (T112 and T113) were previously shown (4) to cause an antigen-independent activation of the cytolytic mechanism of CTL (as evidenced by the induction of nonspecific cytolytic activity). In addition, T11+T3−Ti− NK cell clones are induced by the same antibodies to cause lysis of NK-resistant targets. Collectively, these results indicated that T11 triggering could activate cytotoxic lymphocytes to express their functional programs in the absence of specific antigen recognition via the T3-Ti complex. This raised the possibility that TCAF might modulate cytotoxic effector function as well.

Materials and Methods

Clones. The NK and T cell clones used in this work were isolated as previously reported (4, 5) and their characterization is presented in Table I. In brief, QQ and AA8 are alloreactive cytotoxic T cell clones from two different donors. Clone QQ (4) is specific

Supported by National Institutes of Health grants AI19807, AI21226, and CA40134. R. Siliciano was supported by a fellowship from the Myasthenia Gravis Foundation. R. Schmidt's present address is Abt. Immunologie und Transfusionemedizin, Zentrum Innere Medizin und Dermatologie, Med. Hochschule Hannover, Postfach 610180, D-3000 Hannover G1, West Germany.

1 We elsewhere refer to this same lymphokine as IL-4A.
TABLE I

Characterization of Cytotoxic and Natural Killer Clones

<table>
<thead>
<tr>
<th>Clones</th>
<th>Function</th>
<th>Phenotype</th>
<th>Cytotoxic specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>QQ</td>
<td>CTL</td>
<td>T3+T4-T8+T11+NKH1</td>
<td>HLA-B7</td>
<td>4</td>
</tr>
<tr>
<td>AA8</td>
<td>CTL</td>
<td>T3+T4-T8+T11+NKH1</td>
<td>HLA-Dr7 or Dr4</td>
<td>6</td>
</tr>
<tr>
<td>JT10</td>
<td>NK</td>
<td>T3+T4-T8+T11+NKH1</td>
<td>140kD TNK4</td>
<td>5</td>
</tr>
<tr>
<td>CNK6</td>
<td>NK</td>
<td>T3+T4-T8+T11+NKH1</td>
<td>Non-MHC</td>
<td>5</td>
</tr>
</tbody>
</table>

for the HLA-B7 molecule on the allogeneic EBV-transformed B cell line Laz 156 (HLA genotype: A2, A3, B7, B40, Dr2, and Dr4), while clone AA8 (6) is directed at the HLA class II molecules on the allogeneic B lymphoblastoid line Laz 509 (A2, A25, B13, Bw38, Dr4, and Dr7).

The NK clones (5) were derived from the peripheral blood of the same individual and were selected for their ability to kill K562 target cells without prior immunization. Cytotoxicity of these clones cannot be blocked by mAbs specific for class I or class II MHC products (7). Clone JT10 has been shown to be specific for a cell surface glycoprotein of 140 kD (TNK4) expressed on lymphoid and hematopoietic cells after activation (8). Furthermore, its cytotoxicity is also blocked by antibody to T3. Unlike JT10, the CNK6 clone is not specific for the TNK4 antigen and does not express the T3 surface marker.

Functional Assays. Cytotoxic activity was measured using a standard 51Cr release assay (9) in which effector clones and labeled target cells were mixed at a 1:1-10:1 E:T ratio in a final volume of 0.2 ml. After centrifugation, assay plates were incubated for 4 h at 37°C before removal of aliquots of supernatant for detection of radioactivity.

Lymphokines and mAbs. The mAbs anti-T112 (IgG2a) and anti-T113 (IgG3) were produced and characterized as described elsewhere (2) and were used in ascites form at a 1:100 final dilution. Recombinant human IL-1 (a generous gift of Dr. C. A. Dinarello, Tufts Medical School, Boston, MA) was used at a final concentration of 25 U/ml, a concentration well in excess of that required for optimal activity in a murine thymocyte proliferation assay. rIL-2 and rIFN-γ (Biogen, Cambridge, MA) were used at final concentrations of 1000 U/ml. Partially purified TCAF was obtained as described and used at 4 U/ml (3). Briefly, Th supernatant was subjected to a 90% saturation of ammonium sulfate and the redissolved precipitate was applied to a Sephadex G-100 molecular sieve column. Fractions containing TCAF activity were pooled and concentrated, and subjected to further molecular sieve chromatography on a Sephadex G-50 column. The fractions containing TCAF were again pooled and concentrated, yielding ~35% of the TCAF activity when compared with the original supernatant, but were devoid of IL-2 activity.

Results and Discussion

To determine whether TCAF could induce antigen-independent activation of the cytolytic mechanism, a series of experiments was performed using clonal cytotoxic effector populations. QQ is a representative T3+T4+T8+ cytotoxic T cell clone specific for the HLA-B7 molecule expressed by the B lymphoblastoid line Laz 156. As shown in Fig. 1, in the absence of anti-T11 antibodies or TCAF, this clone will lyse Laz 156 cells but not Laz 509, an EBV-transformed B cell line derived from a donor of a different HLA genotype. As previously reported, the presence of a combination of anti-T112 and anti-T113 antibodies induced clone QQ to lyse the inappropriate target Laz 509. A similar effect was observed with the purified TCAF lymphokine (4 U/ml). In contrast, purified IL-1, IL-2, or IFN-γ could not induce a cytotoxic response against Laz 509 cells. Although not shown, serial dilution of the TCAF material resulted in a dose-dependent diminution in cytotoxic effector function. In addition, no cytotoxicity
FIGURE 1. Lysis of $^{31}$Cr-labeled B lymphoblastoid target cell lines Laz 509 and Laz 156 by the alloreactive cytotoxic T cell clones QQ (A) and AA8 (B). QQ is a CTL clone specific for an MHC class I antigen expressed on Laz 156 and lacking on Laz 509, whereas AA8 is directed to an MHC class II antigen present on Laz 509 (E/T ratio, 1:1). In these experiments, units of lymphokines are as follows: IL-1, 25 U/ml; IL-2, 1,000 U/ml; IFN-γ, 1,000 U/ml; TCAF (IL-4), 4 U/ml. A unit of TCAF is equivalent to the concentration of lymphokine necessary to obtain 50% of the maximum proliferation in a proliferative assay of resting T lymphocytes.

was induced by any of the other three lymphokines, even when doses of recombinant material ranging from 1–1,000 U/ml were assayed in the system.

As shown in Fig. 1B, the T3+T4+T8− cytotoxic T cell clone AA8, which is specific for a class II molecule on Laz 509 but lacking on Laz 156 cells, could kill the latter in the presence of either anti-T11 antibodies or TCAF. Consistent with the above result for the T8+ clone QQ, the other lymphokines assayed in this system again could not induce cytotoxic effector function against the irrelevant target cell. Thus, CTL effector function of both T4+ and T8+ CTL clones is uniquely regulated by either TCAF or anti-T11 antibodies.

The induction of antigen-independent cytotoxicity via the TCAF lymphokine results from the action of the lymphokine on the cytotoxic T cell clones since: (a) TCAF does not induce lysis of target cells in the absence of CTL and (b) target cells do not express the T11 molecules, which is the putative TCAF surface receptor (data not shown). Furthermore, the effect of TCAF could be observed if CTL were pretreated with the lymphokine. Although not shown, it was found that TCAF-induced cytotoxicity, like anti-T11–induced cytotoxicity, was not mediated by clones whose active genetic program lacked the cytotoxic machinery. This is not surprising in view of the fact that prior studies with anti-T11 indicated that cytotoxicity occurs through effector mechanisms virtually identical to those involved in specific cytotoxicity, including calcium requirements, temperature sensitivity, and cell contact dependence (4).

NK cells are operationally defined as those capable of mediating direct cytotoxicity against various target cell types without apparent prior immunization. NK cells are not restricted by the MHC antigens expressed on their target cells. Both T3+T11+ and T3−T11+ NK cells have been defined (5). T11+T8− NK cells
contain Ti alpha and Ti beta mRNA transcripts and express disulfide-linked heterodimers on the cell surface, consistent with the presence of a functional T cell receptor. In contrast, T11⁺T3⁻ NK clones express only truncated 1.0 kb Ti beta transcripts without Ti alpha transcripts and possess no detectable surface Ti protein. Since prior studies showed that Ti beta gene activation precedes Ti alpha gene activation within the thymus (10), the T11⁺T3⁻ NK cells appear to be derived from T lineage precursors. The ability of anti-T11 antibodies (4) to trigger cytotoxic activity from NK clones further supports this notion.

To determine whether TCAF would activate representative NK clones, we examined the non-MHC-restricted T3⁺T11⁺ NK clone CNK6 and the T3⁺T11⁺ NK clone JT10, previously shown to be specific for the non-MHC-restricted target antigen TNK₅₆₂ (a 140 kD glycoprotein that is widely expressed on normal lymphoid and hematopoietic cells after activation). As shown in Fig. 2, both JT10 and CNK6 (Fig. 2, A and B, respectively) efficiently killed the NK susceptible target K562. In contrast, neither clone by itself was cytotoxic for the NK-resistant B lymphoblastoid line Laz 509. The addition of anti-T11 antibody or TCAF, however, induced lysis of the NK-resistant target Laz 509. The ability to induce killing activity from cells that lack a T3-Ti antigen/MHC receptor complex by triggering through the T11 structure, as in the case of CNK6, further substantiates the view that the T11 molecule represents an independent mechanism of cell activation.

What might be the in vivo function of the TCAF-mediated induction of the killing machinery of clones with cytotoxic potential, given that it is independent of antigen specificity? One likely possibility is that TCAF functions as a vector for amplifying cellular immune responses of both effector and regulatory types. In the case of the CTL described here, it is likely that TCAF may be the basis by which the T cell immune system can maintain millions of CTL receptor specificities for various antigens on target cells by virtue of unique Ti alpha-beta V domain combinations, and still provide protective immunity against virus or...
other pathogen infected cells, benign or malignant. While there must exist homeostatic controls to terminate TCAF-mediated cytotoxicity, it is not unreasonable to assume that the lymphokine itself may be functioning exclusively over short distances and facilitates cytotoxicity in infected tissues once antigen-specific Th have been triggered.

In the case of the NK populations, it would appear that the T cell product, like the anti-T11₂ + anti-T11₃ antibodies (4, 11), can facilitate recruitment in several ways. First, TCAF can function to directly induce a given NK clone to express its lytic program. Second, by virtue of the fact that TCAF induces IL-2-R expression on T11⁺ cells (3), and consequently IL-2 responsiveness, the TCAF lymphokine will presumably lead to clonal expansion of these cells. The recent observations regarding IL-2-inducible NK cells (so called LAK cells) (12, 13) are most likely a consequence of such an increase in the number of effector cells expressing IL-2 receptors. Emphasis should be placed, however, on the fact that IL-2, unlike TCAF, does not directly stimulate the cytotoxic program of a given activated CTL (see Fig. 1) or NK clone (data not shown). Furthermore, IL-2 stimulation does not result in alteration of clonal specificity. Additional analysis of the effects of TCAF on heterogeneous peripheral blood T and NK populations and production of neutralizing anti-TCAF antibodies will further elucidate the physiology of TCAF with respect to cytotoxic effector function.

Summary

A 10–12 kD lymphokine, herein termed TCAF, was recently shown to be secreted from Th after crosslinking of their antigen/MHC (T₃-Ti) receptors. TCAF stimulates resting T lymphocyte proliferation via binding to surface components of the T₁₁ pathway. To determine whether TCAF could induce antigen-independent activation of the lytic machinery of cytotoxic cells, the present studies were conducted. In the presence of TCAF, both T₈⁺ MHC-specific and T₄⁺ MHC-specific cytotoxic T cell clones were induced to kill targets, including those lacking the appropriate MHC molecules. This effect was unique to TCAF, since IL-1, IL-2, IFN-γ could not stimulate lytic activity. Furthermore, both T₃⁺T₁₁⁺ and T₃⁻T₁₁⁺ NK clones were triggered to lyse NK-resistant target cells. These findings suggest that TCAF can function in an antigen-independent fashion to amplify cytotoxic effector responses.

The authors would like to thank Ms. Joanne Pratt and Ms. Gail Bartley for their excellent assistance in culture and growth of the clones used in this work.

Received for publication 21 January 1986 and in revised form 12 March 1986.

References


