Efficient Killing of Chronic B-Lymphocytic Leukemia Cells by Superantigen-Directed T Cells

By Anna Carin Wallgren, Roger Festin, Cecilia Gidlöf, Mikael Dohlsten, Terje Kalland, and Thomas H. Törterman

In vitro studies have indicated that chronic lymphocytic leukemia of B-cell origin (B-CLL) is resistant to cytotoxic effector lymphocytes such as natural killer and lymphokine activated killer (LAK) cells. We show here that B-CLL cells are sensitive to Staphylococcal enterotoxin (SE) A-directed T-cell killing. Activation of the target cells by phorbol ester (tetradecanoyl phorbol acetate, [TPA]) greatly enhances their sensitivity to lysis. In SE-dependent cellular cytotoxicity (SDCC), members of the SE superantigen family form a bridge between T cells and target cells expressing major histocompatibility complex class II molecules. Binding of SEA to the T-cell–receptor Vβ region induces a strong cytotoxic capacity and cytokine production. Cells from 9 B-CLL patients were cultured in the presence or absence of TPA and used as targets in a 4-hour SDCC assay using an allogeneic T-cell line as effector. At an effector:target cell ratio 30:1, 70% to 80% of TPA-activated B-CLL cells were killed. Even at the effector:target ratio of 3:1, 47% ± 6% of TPA-activated B-CLL cells were lysed compared with 13% ± 2% of resting cells (P < .001). A T-cell line established from a B-CLL patient killed autologous tumor cells as efficiently as allogeneic effectors. SEA-directed T cells were far more lytic to B-CLL cells compared with LAK cells or lectin (phytohemagglutinin-directed) T cells. Mechanisms of SDCC lysis were investigated. Effector plus target cell supernatants contained high levels of tumor necrosis factor (TNF-α) and interferon-γ, but these supernatants were not directly toxic to B-CLL cells in short term culture. High concentrations of recombinant TNF-α or TNF-β had no lytic effect. Addition of neutralizing anti–TNF-α and anti–TNF-β antibodies into the SDCC assay did not inhibit SEA-directed T-cell killing. TPA-activated B-CLL cells showed a 1.2- to 13-fold increased expression of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1), lymphocyte function-associated antigen (LFA)-1, and LFA-3, whereas expression of HLA class II molecules increased up to 5 times. The expression of CD72, CD40, and BB/1/B7 increased 1.8 to 4.5 times. The role of these surface molecules in SDCC was analyzed in blocking experiments with monoclonal antibodies. Antibodies to ICAM-1, CD18, and HLA-DR abolished the cytotoxicity, and a substantial reduction was seen with antibody to CD72. We conclude that superantigen-directed T cells deliver the most effective cell-mediated killing of B-CLL cells hitherto described. Lysis is dependent on direct cell-to-cell contact and involves ICAM-1, CD18, and CD72 molecules. © 1993 by The American Society of Hematology.

CHRONIC LYMPHOCYTIC leukemia of B-cell origin (B-CLL) is the most common type of leukemia in the western hemisphere. B-CLL cells represent a clonally expanded homogeneous population with a slightly more immature and activated phenotype than that of circulating normal B cells. The disease is difficult to cure with conventional chemoradiotherapy, and patients with advanced B-CLL have a mean survival of 5 to 6 years. Immunomodulatory therapy with cytokines such as interferon-α (IFN-α) has not been effective in B-CLL, although IFN-α is effective in hairy cell leukemia, a closely related disease. In vitro cytotoxicity studies using natural killer (NK) or lymphokine activated killer (LAK) cells as effectors and resting or phorbol ester (tetradecanoyl phorbol acetate [TPA]-activated B-CLL cells as targets showed that B-CLL cells are quite resistant to direct cell-mediated killing.

The Staphylococcal enterotoxins (SE) A, B, C, D, and E, and some related bacterial exotoxins have been termed superantigens because of their ability to stimulate large numbers of human or murine T cells expressing particular T-cell–receptor (TCR) Vβ sequences. Part of the SE molecule has high affinity for major histocompatibility complex MHC class II molecules (particularly HLA-DR, but also DR-DQ and -DP) and is presented to the T cell as an unprocessed antigen. The binding site is located outside the conventional peptide-binding groove. SE interacts with certain TCR-Vβ chain sequences and are the most potent T-cell activators known. CD4+ naive and memory cells and CD8+ cells respond by proliferation, production of cytokines such as interleukin-2 (IL-2), IFN-γ, tumor necrosis factors (TNFs), and generation of strong cytotoxic capacity. SE-directed T cells kill not only HLA-DR+ tumor target cells but also normal DR+ cells such as activated T cells, B cells, and monocytes. Furthermore, it was shown that effector-target conjugate formation and lysis are dependent on cell adhesion molecules. SE-directed T-cell killing was recently termed SE-dependent cell-mediated cytotoxicity (SDCC).

Because of the reported high cytolitic efficiency of the SDCC mechanism, we investigated SEA-directed T-cell killing of primary B-CLL cells in vitro. Preactivated B-CLL cells were more efficiently lysed by SEA-specific T-cell lines than resting B-CLL cells. SDCC was far more efficient than lysis mediated by LAK cells and lectin-directed T cells. Activation of B-CLL cells and acquisition of sensitivity to lysis was paralleled by increased expression of several functionally important cell surface molecules.

MATERIALS AND METHODS
Reagents
SEA and SEB were obtained from Toxin Technology Inc (Madison, WI). Biotinylated SEA was prepared as previously described.
The following fluorescein (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies (MoAbs; specificities in parentheses) and reagents were purchased from Becton Dickinson (Mountain View, CA) and used in two-color fluorescence-activated cell-sorter FACS analysis: anti-Leu-12-PE (CD19), anti-Leu-2-PE (CD8), anti-Leu-3-FITC (CD4), Avidin-FITC, anti-Leu-10-FITC (HLA-DQ), and anti-HLA-DR. The MoAbs IOL58 (anti-lymphocyte function-associated antigen [LFA]-3), anti-CD58, IOL54 (anti-intercellular adhesion molecule-1 [anti-ICAM-1], anti-CD54), and IOT16 (anti-LFA-1, anti-CD1a) were from Immunotech (Mar- selle, France). Tl l-1 (anti-CD2) and 9-49 (anti-HLA-DR) were kindly provided by Dr S. Schlossman (Dana-Farber Cancer Institute, Boston, MA). 1B4 (anti-CD18) was a gift from Dr A. Grönborg (Kabi Pharmacia, Uppsala, Sweden), and BU40 (anti-CD72) and B-B20 (anti-CD40) were from Serotec (Oxford, UK). Anti-BB-1/B7 was kindly provided by Dr E. A. Clark (University of Washington, Seattle, WA). Anti-thyroid stimulating hormone (TSH) was a gift from Dr M. Carlsson (University of Uppsala, Upp- sala, Sweden). Rabbit anti-SEA antibodies were kindly provided by Kabi-Pharma- cia, with 10% fetal calf serum, 1 mmol/L nonessential amino acids, and 1 mmol/L pyruvate (GIBCO, Middlesex, UK) was used as complete medium.

**Cell Lines**

Human peripheral blood mononuclear cells (MNC) were iso- lated from a normal healthy subject by routine density centrifuga- tion. An SEA-reactive T-cell line, SEA-T, was established by stimula- tion of these cells with SEA (1 ng/mL). The cell line was kept for 4,000 rad) BSM B-lymphoblastoid cells and rIL-2 (20 U/mL) in 1 week before use. The T-cell line was greater than 99% CD3+, 47% CD8+, 42% CD4+. BSM and the LAK-cell-sensitive Daudi cell line were grown in log phase in complete medium. Cells were cultured plus TPA and preincubated with or without SEA. The target cells were cultured ± TPA and preincubated with or without SEA. (see above) were harvested and added to target cells from 2 patients (no. 1 and 3). Cytotoxicity was measured in the 4-hour 51Cr-release assay. The target cells were cultured ± TPA as described and preincubated with or without SEA (10 ng/mL).

**Cytotoxicity Assays**

*SE-directed cytotoxicity.* Cell-mediated cytotoxicity was measured at various effector:target cell ratios (30:1, 10:1, 3:1, 1:1) in a standard 4-hour 51Cr-release assay and expressed as % specific lysis = (experimental cpm - background cpm/maximal cpm - background cpm). Target cells were labeled for 2 hours with 51Cr (250 μCi/1 x 106 cells). Thereafter, the cells were preincubated with or without SE (10 ng/mL) for 30 minutes at room temperature, washed in complete medium, and seeded in v-bottomed microtitre plates at a concentration of 2.5 x 105 cells/well. Indicated numbers of effector cells were added in 0.2 mL of complete medium. Super- natants were collected, and the released 51Cr was measured in a γ counter (LKB-Wallac 1282, Uppsala, Sweden). Spontaneous re- lease was estimated by incubation of target cells in medium alone, and maximum release by resuspending the cells with 0.1% Tween-20 (Sigma, St Louis, MO).

**Lectin-mediated cytotoxicity.** Cytotoxicity was measured as above at the same effector:target cell ratios and with 1 μg/mL PHA added directly into the assay. Lectin-mediated cytotoxicity was tested both in the absence and presence of SEA or SEB.

**Cytotoxicity mediated by SDCC supernatants.** Supernatants from effector- and target-cell combinations cultured 4 hours or 20 hours (see above) were harvested and added to target cells from 2 patients (no. 1 and 3). Cytotoxicity was measured in the 4-hour 51Cr-release assay. The target cells were cultured ± TPA as described and preincubated with or without SEA (10 ng/mL).

**Blocking of TNF-α and TNF-β during SDCC.** Rabbit anti-human TNF-α and anti-human TNF-β antibodies (diluted 1:100) were added directly into the SDCC assay. At this dilution, each antisem neutralized greater than 7,000 U/mL of rTNF-α and rTNF-β, respectively. Rabbit anti-SEA antibodies were kindly provided by Kabi-Pharma- cia. RPMI 1640 (Flow Laboratories, Irvine, UK) supplemented with 10% fetal calf serum, 1 mmol/L nonessential amino acids, and 1 mmol/L pyruvate (GIBCO, Middlesex, UK) was used as complete medium.

**Cell Cultures**

 Freeze-stored mononuclear cells from patients with untreated classic B-CLL (95% to 99% monoclonal CD19+ cells) were thawed, cultured for 3 days with or without TPA (1.6 x 10-7 mol/L) in complete medium, and used as targets in the SDCC assay. Cells were also stained with MoAbs and phenotyped by FACS analysis (see below). MNC from a normal healthy subject were stimulated with 100 U/mL of rIL-2 for 3 days to obtain LAK cells. These LAK cells lysed 68%, 48%, and 28% of Daudi cells at the corresponding effectortarget ratios 30:1, 10:1, and 3:1, respectively. To study a possible cytotoxic “passive bystander effect” of SDCC supernatants on B-CLL targets, the effector SEA-T line was cocultured with targets from 2 patients (no. 1 and 3). The target cells were stimulated as above and preincubated with or without SEA (10 ng/mL). Two different effector:target cell ratios (30:1 and 3:1) were used. SEA-coated or uncoated effector or target cells cultured alone were used to produce control supernatants. The different supernatants were collected after 4 hours and 20 hours of culture and assayed for cytokines in EASIA. To analyze direct cytotoxicity, supernatants were added to different target cells in a 4-hour 51Cr-release assay.

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Analysis of Cell Phenotype

Double-stained cells were analyzed on a FACScan (Becton Dickinson, San Jose, CA) 5 parameter flow cytometer equipped with a 15-mW Argon ion laser emitting at 488 nm according to standard settings. Green fluorescence (FITC) was collected through a 530/30-nm bandpass filter, and red fluorescence (PE) through a 585/42 filter. Ten thousand cells were acquired and evaluated with the Consort 30 software. Appropriate isotypic control-MoAbs were used to estimate the level of nonspecific surface binding. BSM cells were used as a positive control for binding of SEA-biotin and for expression of MHC class II and adhesion molecules.

RESULTS

Specificity of the Effector T-Cell Line

To study the sensitivity of B-CLL cells to SE-directed T-cell killing, an SE-reactive T-cell line was established from a healthy donor by repeated stimulation with SEA-coated lymphoblastoid BSM cells. This T-cell line showed strong cytotoxicity against SEA-coated preactivated B-CLL cells but not against uncoated cells. The cytotoxicity against B-CLL cells preincubated with SEB was low, showing that the T-cell line was specific for SEA (Fig 1).

Lytic Efficacy of SEA-Directed T Cells Compared With Lectin-Directed T Cells and LAK Cells

Lectin-dependent killing of B-CLL cells. SEA-directed killing was also compared with lectin-mediated cytotoxicity. PHA was added directly into the assay with uncoated, SEA-coated, or SEB-coated target cells, respectively. As shown in Fig 1A and B, PHA mediated a similar but less effective cytotoxicity against both resting and TPA-activated CLL cells. The SEA-directed killing was not improved by the addition of PHA during the assay. Identical results were seen with tumor cells from 2 other patients.

Role of target cell preactivation in SDCC. The impact of phorbol ester pretreatment of target cells is shown in Figs 2A and B. Two individual B-CLL clones were studied. Unstimulated cells from 1 patient (Fig 2A) were totally resistant, while 51% of the TPA-activated cells were lysed at a low E:T ratio of 3:1. Resting cells from the second patient (Fig 2B) showed some sensitivity in SDCC, whereas the preactivated cells were lysed in a dose-response-dependent fashion (24% compared with 55% at an E:T ratio of 30:1).

Thus, B-CLL cells had to be activated to be efficiently killed by SEA-directed T cells.

A T-cell line similarly established from B-CLL patient no. 1 lysed resting or TPA-activated autologous tumor cells as efficiently as the allogeneic effector cells (Fig 2C).

B-CLL cells cultured with or without TPA are resistant to LAK cells. Normal blood mononuclear cells were cultured in the presence of 100 U rIL-2/mL for 3 days, and the cytotoxic capacity of the generated LAK cells was tested against B-CLL cells from several patients (Fig 3A) and against the standard LAK cell target Daudi. The latter cell type was highly sensitive (see Materials and Methods). To analyze whether activation could influence the sensitivity to LAK-mediated lysis, B-CLL cells were cultured for 3 days with or without TPA. At E:T ratio 10:1, LAK cells were able to kill 12% ± 4% unstimulated and only 4% ± 2% TPA-stimulated B-CLL cells. Thus, TPA did not significantly alter the sensitivity of B-CLL cells to LAK-cell killing. Altogether, both resting and TPA-activated B-CLL cells were highly resistant to lysis by LAK cells.

Comparison of the efficacy of SEA-specific T cells and LAK cells to kill different targets. Figure 3 compares the efficacy of LAK cells and the SEA-specific allogeneic T-cell line to lyse the uncoated or SEA-coated resting or activated B-CLL cells from several patients. B-CLL cells from 9 patients were tested as targets and the SEA-reactive T-cell line was used as effector. Target cells were cultured in the presence or absence of TPA for 3 days and, thereafter, coated with SEA. Unstimulated B-CLL cells showed low or no sen-
Superantigen-Mediated Killing of B-CLL

Fig 2. SEA-mediated cytotoxicity against cells from B-CLL patients. MNC from patients no. 3 (A) and no. 1 (B and C) were cultured with or without TPA for 3 days and incubated for 30 minutes in room temperature with or without 10 ng/mL SEA before assay. The allogeneic SEA-T-cell line was used as effector in (A) and (B). An SEA-T-cell line derived from B-CLL patient no. 1 was used against autologous tumor cells in (C). Cytotoxicity against uncoated or SEB-coated targets was close to 0% in each case (data not shown).

Fig 3. SEA-mediated T-cell killing of cells from several B-CLL patients. Lytic efficacy of different effector cells and impact of target cell activation (data as mean ± SEM). (A) IL-2 activated LAK-cell effectors against resting (-TPA) or activated (+TPA) B-CLL cells. (B) LAK-cell effectors against SEA-coated resting or activated B-CLL cells, and (C) the SEA-T-cell line as effector against SEA-coated resting or activated B-CLL cells. Data were compared using the paired t-test. *P < .05, **P < .01, and ***P < .001.
Fig 4. Flow cytometric analysis of the expression of adhesion molecules and HLA class II molecules on resting and TPA-activated B-CLL cells and their ability to bind SEA. Cells from 4 patients (no. 1, 2, 3, and 4) were analyzed. The changes in mean fluorescence intensity are shown. The expression on resting B-CLL cells is presented as 1.0 (horizontal bar) for comparison with activated cells. As a control for nonspecific binding, rabbit antimouse F(ab)2-FITC was used alone or in combination with irrelevant mouse IgG of the same isotype. No binding was seen with either of the latter combinations (data not shown).

TPA-Induced B-CLL Cells Show Increased Expression of Several Cell Surface Molecules and Binding of SEA

Phorbol-ester–induced phenotypic changes in CD19+ B-CLL cells from 4 patients were studied by two-color FACS analysis using MoAbs to the MHC class II molecules HLA-DP, -DQ, -DR, and the adhesion molecules ICAM-1, LFA-3, LFA-1, and CD18. The capacity of B-CLL cells to bind SEA was also measured. Furthermore, the expression of CD72, BB-I/B7, and CD40 was quantified in 2 patients (Fig 4). Untreated B-CLL cells showed high levels of CD40 and MHC class II molecules but showed low levels of all adhesion molecules analyzed. After induction of the tumor cells with TPA, a 1.2- to 13-fold increase in expression of ICAM-1 (relative fluorescence intensity for TPA-activated cells in arbitrary units [median and range], 182, 52 to 312), LFA-1 (105, 60 to 430) and LFA-3 (194, 110 to 266) was registered (Fig 4). Both the percentage of positive cells (data not shown) and mean cellular fluorescence intensity increased. The expression of MHC class II molecules and binding of SEA increased only slightly as to percent positive cells and up to 5 times with respect to fluorescence intensity (for HLA-DR median intensity 387, 98 to 630). B-CLL cell expression of CD72 (31, 46), CD40 (247, 423), and BB-I/B7 (20, 36) increased 1.5 to 4.5 times in 2 patients studied. An attempt was made to correlate the relative increase in expression of the studied cell surface antigens with sensitivity to lysis in SDCC. No such correlation was observed.

SEA-Directed Killing of B-CLL Cells is Blocked by MoAbs to HLA-DR, ICAM-1, CD18, and CD72

MoAbs against HLA-DR (ascites diluted 1:300 and 1:500) were added to the SDCC assay using TPA-activated B-CLL target cells preincubated with increasing concentrations of SEA (0.1, 1, and 10 ng/mL). The cytotoxicity was completely abolished by both concentrations of MoAb, when targets coated with the lower concentrations of SEA were used. Using anti–HLA-DR MoAb diluted 1:500 and targets preincubated with a high concentration of SEA (10 ng/mL), 82% of the cytotoxicity was inhibited (Fig 5). Further blocking experiments were performed with MoAbs to CD72, CD40 and the adhesion molecules ICAM-1, CD18, and LFA-3 to establish the significance of these molecules in SDCC against TPA-stimulated B-CLL cells (Fig 6). A complete inhibition of SEA-mediated cytotoxicity was seen with 20 μg/mL of anti–ICAM-1 MoAb in 1 of 2 patients tested (Fig 6A). A similar dose-dependent decrease in percent cytotoxicity was observed with anti-CD18 MoAb (Fig 6B). Using MoAb against CD72, the cytotoxicity decreased from 62% to 11% (Fig 6C). Anti–LFA-3, anti-CD40, and anti-BB-1/B7 MoAbs did not interfere with SDCC against any of the target cell populations (Fig 6D). In a control experiment with anti-CD2 (T11-1 ascites 1:400) alone or in combination with anti–LFA-3, we observed no inhibition of cytotoxicity by SEA-T against TPA-activated B-CLL cells (data not shown). An irrelevant control MoAb anti-TSH did not inhibit SDCC (Fig 6D). Thus, the surface molecules ICAM-1/ LFA-1 and CD72/CD5 play important roles in SEA-directed T-cell killing of B-CLL cells.

Cytokine Content and Cytotoxic Effect of SDCC Assay Supernatants

The production of TNF-α and IFN-γ by the SEA-T target cell line was measured by EASIA kits. The spontaneous release of TNF-α ranged from 14 to 18 pg/mL, and the release for IFN-γ ranged from 0.8 to 1.1 IU/mL. In the presence of SEA or SEA-coated targets, the production of TNF-α and
Fig 6. MoAb against ICAM-1, CD18, and CD72 inhibit SEA-mediated T-cell lysis of TPA-induced B-CLL cells. Cytotoxicity of the SEA-T-cell line was measured against targets from 2 patients (C, no. 1; 0, no. 2) in the presence of various concentrations of MoAbs to (A) ICAM-1, (B) CD18, and (C) CD72. Target cells were pretreated with 10 ng/mL SEA, the effector:target ratio was 10:1 and MoAbs were added at the initiation of the assay. (D) MoAbs against BB-1, CD40, LFA-3, and TSH (nonspecific control) did not interfere with the SDCC mechanism (mean values of 2 patients). The cytotoxicity in the absence of SEA was close to 0% with or without MoAbs.

IFN-γ increased 12.3 to 59 times and 4.6 to 7.5 times, respectively. We tested the “passive bystander” effect, ie cytotoxicity of these supernatants against TPA-stimulated B-CLL cells ±SEA in the absence of effector cells. No toxicity was observed as determined by the 4-hour chromium release assay (data not shown).

Effect of Addition or Blocking of TNF-α and TNF-β

High amounts of rTNF-α (600,000, 60,000, and 6,000 U/mL) or rTNF-β (140,000, 14,000, and 1,400 U/mL) were added to resting or TPA-activated target cells (coated or not coated with SEA) from 2 patients (no. 1 and 2) in the absence of effector cells. No apparent cytotoxicity could be detected in any combination using the 4-hour chromium release assay (data not shown).

High local concentrations of TNF in the vicinity of the effector-target conjugate might be important in cytotoxicity. Therefore, neutralizing rabbit anti-TNF-α and anti-TNF-β polyclonal antibodies (final dilutions 1:100) were added simultaneously into SDCC assays containing SEA-T effector cells and TPA-activated SEA-coated target cells from 2 patients (no. 1 and 2). No inhibition of chromium release was observed (Fig 7). Normal rabbit serum did not interfere with SDCC, but, as expected, rabbit anti-SEA had a blocking effect on the ability of effectors to mediate cytotoxicity.

DISCUSSION

Patients with B-CLL have a great expansion of circulating monoclonal B cells that outnumber residual T and NK cells. Several phenotypic and functional abnormalities in T and NK cells from B-CLL patients have been shown. These include reduced proportions of T-helper and T-suppressor inducer cells, increased phenotypic activation of T and NK cells, depressed responses to T- and B-cell mitogens, and depressed NK and antibody-dependent cell-mediated cytotoxicity functions.20-26 A recent study by Foa et al6 showed that LAK cells generated from B-CLL patients were less efficient killers than LAK cells from healthy subjects. These investigators further showed that B-CLL cells were resistant to lysis by normal or autologous LAK cells. Immunomodulatory therapy with IFN-α and, to some extent, with IL-2 has been tried in B-CLL but proved to be ineffective at least in the more advanced stages of disease.3,4,27 These disappointing clinical results may reflect not only the in vitro resistance of B-CLL cells to lysis by NK/LAK cells but also the defective in vitro generation of LAK activity in B-CLL.6

Fig 7. Neutralizing antibodies to TNF do not inhibit SEA-mediated T-cell lysis of TPA-activated B-CLL cells. Rabbit anti-TNFα and anti-TNFβ (final dilutions 1:100; neutralizing capacity > 7,000 U/mL each) were present in the SDCC assay with allogeneic SEA-T-line cells as effectors against SEA-coated TPA-activated targets (patient no. 2) at ratios 30:1 and 3:1. Normal rabbit serum and rabbit anti-SEA served as negative and positive controls, respectively. Cytotoxicity against uncoated targets was near 0%. Similar results were obtained with CLL target no. 1 (not shown).
Our present series of experiments show that phorbol ester-activated B-CLL cells become highly sensitive to killing by SEA-directed T cells, whereas unstimulated B-CLL cells proved to be relatively resistant to lysis via the SDCC mechanism. Superantigens of the SE family have the capacity to bind to MHC class II molecules on the target cell, and T cells conjugate to the targeted SE molecule via restricted families of TCR Vβ chains. Large proportions of T cells are activated by SEA and show strong cytotoxicity along with release of cytokines including TNFs, IFN-γ, and IL-2. TNF-β seems to be one important cytotoxic effector molecule. SEs share the superantigen properties with the murine mammary tumor virus (MMTV)-encoded minor lymphocyte stimulating antigens in mice.

The present results show that SEA-directed T cells kill preactivated B-CLL tumor cells more efficiently than lectin-directed T cells, whereas LAK cells are virtually ineffective in this regard. This emphasizes the similarity between SEA- and lectin-mediated killing, both of which use a T-cell–activating “linker” molecule between the effector and target cell. Further evidence for the need of direct cell-to-cell contact in SDCC comes from our studies with culture supernatants. The SEA-directed effector T-cell lines produced considerable amounts of both TNF-α and IFN-γ when cocultured with SEA-coated B-CLL cells. However, such supernatants were not toxic to B-CLL targets in the 4-hour chromium release assay. Furthermore, addition of very high concentrations of rTNF-α and rTNF-β did not mediate cytolysis, and neutralization of spontaneous TNF with antibody did not inhibit SDCC. Taken together, these observations speak against an important role for TNF as a mediator of lysis in SDCC of B-CLL. Preliminary observations indicate that effector T-cell lines express high levels of mRNA for perforin, a molecule mediating direct T-/NK-cell killing of targets.

In cytotoxic T-cell– and NK-/LAK-cell–mediated killing, complementary adhesion molecules on the effector and target cells, respectively, have been shown to enhance conjugation strength and improve lytic efficiency. These adhesion molecules include ICAM-1/LFA-1, LFA-3/CD2, and CD28/BB-1. Therefore, we compared the density of adhesion and MHC class II molecules on resting and TPA-stimulated B-CLL cells to study whether differences in their expression could account for changes in target cell sensitivity. Phorbol-ester activation was accompanied by a 1.2- to 13-fold increase in fluorescence intensity for ICAM-1, LFA-1, and LFA-3 and by a slight increase in expression of class II molecules. The latter was paralleled by an increased binding of SEA. To evaluate the role of surface molecules in SDCC, several experiments were performed using blocking MoAbs. Antibody to HLA-DR inhibited lysis most probably by abrogating the ligation of effector T-cells to the SEA-target cell complex. Furthermore, blocking with anti-ICAM-1 and anti-CD18 MoAb completely abolished B-CLL cell lysis, indicating that the ICAM-1/LFA-1 adhesion molecules play a crucial role in SEA–T-cell–mediated killing of B-CLL cells. However, antibody to LFA-3 and/or anti-CD2 did not interfere with SEA–T-cell–mediated killing of B-CLL cells. In contrast, our earlier results with LFA-3/DR cotransfected CHO target cells showed that blocking MoAbs to either molecule completely inhibits SDCC. The reason why SEA–T-cell–mediated killing of B-CLL cells is critically dependent on ICAM-1/LFA-1 but not of CD2/ LFA-3 or CD28/BB-1 is not clear. These adhesion molecules were expressed with comparable density on the surface of TPA-activated B-CLL target cells. However, the LFA-1 molecule has been shown to possess transmembrane signaling properties, as detected by phosphoinositol hydrolysis and the rise in intracellular Ca++, suggesting that LFA-1 provides costimulatory signals to T cells through ICAM-1 during specific antigen-driven responses. Furthermore, Fischer et al recently showed that LFA-1 delivers a critical costimulatory signal to naive T-helper cells when triggered by SEA.

The expression and engagement of the BB-1/B7 and CD40 surface antigens in SEA–/T-cell–mediated lysis of B-CLL cells was investigated. BB-1 is a B-cell–specific activation antigen, and its natural ligand was recently shown to be CD28, a cytokine regulating T-cell molecule. The expression of BB-1/B7 on B-CLL cells increased after TPA induction, but blocking experiments with anti-BB-1 indicated that this molecule is not involved in SDCC of B-CLL cells. However, we did not have the opportunity to study the effects of MoAb to CD28. CD40 is a type I integral pan-B-cell membrane protein, and ligation of the molecule with antibody induces homotypic adhesion, proliferation, and differentiation. B–B-cell aggregation mediated through CD40 is secondary to induction of the ICAM-1/LFA-1 system. The murine natural ligand (CD40L) for CD40 was recently shown on the surface of activated T cells. TPA-induced B-CLL cells had increased surface expression of CD40. However, blocking antibodies to the molecule had no apparent effect on SDCC.

The involvement of the pan-B–cell surface molecule CD72 in SEA-dependent T-cell–mediated lysis of B-CLL cells was investigated. Preactivation of B-CLL cells with phorbol ester increased their sensitivity to lysis and increased the surface expression of CD72 as well. Furthermore, antibody to CD72 almost completely blocked lysis of B-CLL cells, indicating the importance of this molecule in SDCC. CD72 is a pan-B-cell Ca++-dependent type II integral protein with homology to CD23. Antibody ligation of CD72 promotes entry to cell cycle, upregulates HLA class II, and enhances T-cell–dependent differentiation. The natural ligand for CD72 is CD5, a molecule present on T cells mediating costimulatory signals similar to CD2 and CD28. Physiologic activation of CD72 may, therefore, involve heterophilic T–B interaction. In fact, our present findings substantiate T–B cell bridging through CD72–CD5 and indicate an important role for these molecules in SDCC of B–cell tumors. In contrast to most normal B cells, B–CLL cells express high levels of CD5 on their surface, and TPA-treatment does not downregulate CD5 expression. Thus, homologous B–B (CD5–CD72)–cell interaction could possibly contribute to autocrine growth of B–CLL cells and may also play a role in TPA-induced aggregation of the cells in vitro. Further studies on the CD72/CD5 interaction are warranted.
Although the present results clearly show an important role for ICAM-1, CD18, and CD72 molecules in SEA-directed T-cell-mediated lysis of B-CLL, we were unable to show a positive correlation between cellular expression and sensitivity to lysis. Thus, other surface molecules may be pivotal. In the mouse, cytotoxic T-cell-mediated programmed cell death, or apoptosis, involves the ligation of the apoptosis-inducing Fas molecule on the target cells. The human equivalent molecule APO-1 was recently cloned and included the TNF/NGF receptor superfamily. Studies of the APO-1 molecule should be considered in clarifying the cytotoxic mechanisms of SDCC.

Adhesion molecules on various cell types are susceptible to modulations by several physiologic factors, and Rousset et al. showed that IL-4 may induce expression of certain adhesion molecules on B-cell lines. It is not excluded that SEA/T-cell-mediated killing could be useful in purging of B-CLL cells in vitro, because preliminary results have shown that IL-4, IFN-γ, and TNF-α also modulate adhesion molecules on B-CLL cells (data not shown). However, it would be desirable to use B-cell-specific target structures instead of MHC class II molecules, because the latter are widely distributed among many normal cell types. Dohlsten et al. showed that the target-cell specificity of SEA can be redirected by conjugating the molecule to suitable MoAbs with retained T-cell-activating properties. Recently, recombinant fusion proteins between SEA and MoAbs were constructed that had a significantly reduced binding to MHC class II antigens facilitating in vivo application. B-CLL might be an interesting target for the exploration of the clinical usefulness of similar hybrid molecules based on MoAbs to pan-B-cell surface structures such as CD19 or CD20.

REFERENCES
9. Mollick JA, Cook RG, Rich RR: Class II MHC molecules are specific receptors for Staphylococcus enterotoxin A. Science 244:817, 1989


Efficient killing of chronic B-lymphocytic leukemia cells by superantigen-directed T cells

A Wallgren, R Festin, C Gidlof, M Dohlsten, T Kalland and TH Totterman