Dependency on Intercellular Adhesion Molecule Recognition and Local Interleukin-2 Provision in Generation of an In Vivo CD8+ T-Cell Immune Response to Murine Myeloid Leukemia

By M.W. Boyer, P.J. Orchard, K.B. Gorden, P.M. Anderson, R.S. McLvor, and B.R. Blazar

The immune response to a murine myeloid leukemia (cell line C1498) was studied in vitro and in vivo. Natural killer (NK) cells and CD8+ cytotoxic T lymphocytes (CTL) were shown to lyse C1498 in vitro through the binding of leukocyte function antigen-1 (LFA-1) on effectors and intercellular adhesion molecule-1 (ICAM-1) and ICAM-2 on C1498 target cells. However, the ability of nonimmunized mice to resist an in vivo challenge of a low dose (104) of C1498 was NK-cell, but not T-cell dependent. The failure of T cells to participate in the immune surveillance of a low leukemia burden appeared, in part, because of a lack of expansion of leukemia reactive CTL precursors (CTLp). Leukemia reactive CTLp frequency estimations in naive and leukemia bearing mice were not significantly different (range, 1:20,600 to 1:74,000) in contrast to immunized mice (range, 1:1,400 to 1:4,400). Leukemia reactive CTLp could be expanded to a level that could apparently mediate in vivo immune surveillance of 106 leukemia cells by injection of irradiated leukemia cells intraperitoneally (IP) or subcutaneously (SC), but not intravenously (IV). However, IV injection of 106 live leukemia cells engineered to secrete interleukin-2 (IL-2) resulted in systemic immunity mediated primarily by CD8+ T cells. We conclude that NK cells can mediate immune surveillance of a low leukemia burden. CD8+ CTL-mediated immune surveillance can eliminate a higher leukemia burden than NK cells, but requires T-cell help, which can be delivered by local IL-2. Both NK and CTL-mediated immune surveillance of C1498 murine myeloid leukemia is dependent on recognition through the LFA-1:ICAM adhesion pathway.

© 1995 by The American Society of Hematology.

From www.bloodjournal.org by guest on November 16, 2017. For personal use only.
moderate dose of leukemia overcomes this mechanism of immune surveillance. The critical escape mechanism for this AML cell line appears to be defective CTL expansion in vivo, which can be circumvented either when putative leukemia-associated antigens are provided in sufficient quantities to sites of resident host antigen presenting cells (APCs) or when the leukemia cells are genetically engineered to provide IL-2. Because immunotherapeutic approaches are currently being used for the treatment of AML, these experiments, which are focused on the mechanisms by which AML cells escape immune surveillance in the host, could have important clinical ramifications.

MATERIALS AND METHODS

Mice. Female C57BL/6-Ly5.2 mice (H-2b), age 8 to 12 weeks old, were used for all experiments, and were obtained from the National Institutes of Health (Bethesda, MD). Mice were handled in accordance with the University of Minnesota Research Animal Resources guidelines.

Cells. The cell line C1498, derived originally from a female C57BL/6 mouse (H-2b) was obtained from the American Type Culture Collection (ATCC), Rockville, MD, and was grown in RPMI 1640 (GIBCO BRL, Grand Island, NY) supplemented with 10% heat inactivated fetal bovine serum (Intergen, Purchase, NY), 2 mM/L L-glutamine, 100 mg/mL streptomycin (GIBCO), 100 U/mL penicillin (GIBCO), 0.025 mg/mL fungizone (GIBCO), 1% minimal essential medium amino acids solution (GIBCO), 10 mM/L HEPES (GIBCO), 1 mM/L sodium pyruvate (GIBCO), and 50 mM/L 2-mercaptoethanol (Sigma, St Louis, MO) (referred to as CM). After initial expansion, a large stock of frozen cells was created so that individual experiments always utilized a new vial from frozen stock. The cell line FBL (erythroleukemia, H-2b) was a kind gift of Dr Martin Cheever (University of Washington, Seattle, WA) and was grown in CM. The cell lines EL-4 (T-cell leukemia, H-2b), YAC (lymphoma, H-2b), and P815 (mastocytoma, H-2b) were obtained from ATCC and grown in CM.

Monoclonal antibodies (MoAbs) and flow cytometry analysis. The following MoAbs were used for flow cytometry analysis: anti-H-2 D (28-8-6), anti-I-A* (AF6-120.1), anti-Mac-3 (M/84.6.34), anti-ICAM-1 (3E2), anti-CD4 (RM-4-5), anti-CD8a (53-6.7), anti-NK1.1 (PK3a), anti-gran-1 (RA3-8C5), anti-Mac-1 (M1/70.15.11), anti-B7-2 (GL1), all from Pharmingen, San Diego, CA; anti-Fc type II receptor (2.4G2, ATCC), anti-p5E (372, ATCC), anti-ICAM-2 (IC2-34, kindly provided by Dr Horng Xu, University of Connecticut, Farmington), anti-H-2 K* (EH144, kindly provided by Dr T.V. Rajan, University of Connecticut), anti-Ly 5.1 (104-2, provided by Dr Ule Hammerling, Memorial Sloan Kettering Cancer Research Center, New York, NY), anti-Ly 5.2 (A20-1.7, provided by Dr Hamerling), LFA-1 (FD441.8, provided by Dr Frank Fitch, University of Chicago, Chicago, IL), anti-B7-1 (MB7-07, provided by Dr Gary Gray, Repligen Corp, Boston, MA), anti-pre-B (AA4.1, provided by Dr John McKearn, Monsanto Corp, St Louis, MO), anti-Thy 1.2 (30-H12, provided by Dr David Sachs, Massachusetts General Hospital, Cambridge, MA), anti-CD3 (145-2C11, provided by Dr Jeffrey Bluestone, University of Chicago). The MoAbs used in vivo, anti-CD4 (GK1.5, ATCC), anti-CD8 (2.43, ATCC), anti-NK1.1 (PK3a, ATCC), and anti-LFA-1 (FD441.8, ATCC) were grown as ascites in nude mice for use in vivo. Lots of MoAbs were titrated in vivo 6 to 7 days after intraperitoneal injection (IP) of 2×10⁶ cells*/mouse and both crude and purified antibody were tested either by flow cytometry with greater than 95% depletion of CD4⁺ or CD8⁺ splenocytes; or for titer of LFA-1 and NK1.1 antibodies, with greater than 95% abrogation of NK cell lysis of the NK sensitive target YAC (ATCC) was utilized. Flow cytometry was performed on a FACScan (Becton Dickinson, Mountainview, CA) with proper irrelevant control MoAbs or secondary MoAbs, if antibodies were unconjugated to fluorescein isothiocyanate (FITC) or phycoerythrin (PE). Forward and side scatter settings were set to exclude dead cells/debris and 10,000 events acquired for each analysis.

In vivo lymphocyte subset depletions. Anti-CD4 (GK1.5), anti-CD8 (2.43), anti-NK1.1 (PK3a), and anti-LFA-1 (FD441.8) MoAbs were generated and tiered as described above. Animals were then injected IP (with twice the minimal dose that gave maximum depletion or inhibition) on day −2 and then weekly thereafter for a total of six doses.

Reverse transcriptase assay. Reverse transcriptase activity of cell supernatants was tested by incorporation of radioactive dTTP using a poly(rA) template and subsequent counting of radioactivity on a scintillation counter as previously described. IL-2. Human recombinant IL-2 for in vitro experiments was a kind gift of the Cetus Corporation (Emeryville, CA) with a specific activity of 18×10⁶ IU/mg. IL-2 liposomes were prepared as previously described. Measurement of IL-2 was performed by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN) according to manufacturer’s directions. All IL-2 concentrations are expressed in terms of international units (IU).

Effector cell generation and CTLp frequency analysis. Activated NK cells were generated by injection of 150 μg of polyinosinic-polycytidylic acid (poly I: poly C, Sigma) IP 1 day before harvesting splenocytes. LAK cells were generated by incubation of splenocytes with IL-2 in CM, 1,000 IU/mL for 5 days. CTL were generated by first immunizing mice subcutaneously (SC) with 10³ irradiated C1498 (10,000 rad) and harvesting splenocytes or draining lymph node cells 1 week later, then incubating with irradiated C1498 at a responder to stimulator ratio of 20:1 for 5 days in CM. Cytotoxicity of effectors was assessed by a standard 4-hour chromium release assay, performed in triplicate. Cytotoxicity was determined by the formula: % cytotoxicity = 100 × (exp mean cpm − spontaneous release mean cpm)/(maximal release mean cpm − spontaneous release mean cpm). The ratio of maximum chromium release to spontaneous release was always greater than 4:1. CTLp frequency analysis was accomplished by performing limiting dilution of responding cells (six dilutions) at 30 replicates in 96-well microplates and incubating for 9 days with irradiated C1498 (10⁶/well), irradiated feeder splenocytes (10⁵/well) and IL-2 20 IU/mL. Cytotoxicity was assessed in each well by addition of chromium-labeled C1498 (1,000/well), with an arbitrary cutoff for positivity of 3 standard deviation above mean spontaneous chromium release. Using Poisson distribution statistics according to the method of Taswell and with the aid of a computer program, the likelihood of a single hit was confirmed, and a frequency estimate calculated. Confidence intervals (95% Cl) were obtained based on the chi-square minimization and maximum likelihood methods to compare the CTLp frequencies between groups. Comparisons were made only for frequency estimations performed at the same time under the same conditions.

In vitro blocking of cytotoxicity. Saturating concentrations (100 μg/10⁶ cells) of the indicated MoAbs were incubated with either the effector cells or chromium labelled leukemia targets and then bound MoAb washed free before a standard chromium release cytotoxicity assay.

IL-2 gene transduction. The human IL-2 cDNA transducing retroviral vector LIL-2SN construction and packaging has been previously described in detail. For transduction of C1498, viral supernatants of either the control neomycin vector LN or LIL-2SN were used to transduce C1498 at a multiplicity of five colony forming units per C1498 in the presence of 8 μg/mL polybrene for 24 hours. After another 24 hours, the cells were bulk selected in the neomycin analog G418 (GIBCO). Cells were then placed into limiting dilution with G418 (1 mg/mL) to isolate clonal populations and these tested.
for IL-2 production. A line with highest IL-2 production was chosen for study.

Statistical analysis. The Kaplan-Meier product-limit method was used to assess the survival of mice. The log-rank statistic was used to test differences between groups.

RESULTS

Characterization of C1498 leukemia. C1498 leukemia has previously been characterized histologically as myelomonocytic, which we confirmed by demonstration of expression of a granulocyte marker (gran-1) and two different macrophage markers (Mac 1, Mac 3). C1498 lacked expression of T cell, pre-B cell, and NK cell markers as expected, as well as FcR type II (data not shown). This cell line is uniformly positive for the pan-hematopoietic (CD45) marker Ly 5 and expresses the Ly5.1 allele, allowing the use of this antibody for analysis of leukemia burden in C57BL/6-Ly5.2 congenic mice differing only at the Ly 5 allele.

We found that C1498 is negative for the p15 envelope protein expressed by most murine leukemia retroviruses. Furthermore, reverse transcriptase activity was lacking in this leukemia, while present in a known retrovirally induced murine leukemia FBL (data not shown). Given the reported spontaneous nature of this leukemia and the lack of any evidence for retroviral infection, it is unlikely that a murine leukemia retrovirus is involved in the pathogenesis of this particular leukemia.

With respect to cell surface determinants, which may be involved in an immune response, C1498 is MHC class I positive, but class II negative. Importantly, this leukemia is negative for the costimulatory molecules B7-1 and B7-2. C1498 expresses both ICAM-1 and ICAM-2 on C1498. There is a high level of expression of LFA-1, which most hematopoietic cells express.

C1498 leukemia is sensitive to NK and specific CTL lysis in vitro. To study the interaction of the host immune system with C1498, we first chose to generate lymphocyte effectors in vitro and test their ability to lyse C1498 in a standard chromium release assay. Figure 1A illustrates that either in vivo poly Ipoly C (150 µg/mouse IP) 1 day previously or mice that had been immunized 1 week previously with irradiated C1498, and cytolyis of C1498 was measured as described in Materials and Methods. E:T indicates the effector to target ratio. (B) Draining LN cells were harvested from mice depleted of NK cells and immunized with irradiated C1498, and stimulated in vitro with irradiated C1498. Cytolysis was measured against C1498 or FBL (1A). Data shown are representative of three experiments.

Fig 1. NK and CTL lysis of C1498 in vitro. (A) Splenocytes were harvested from mice (two/group) that received either poly Ipoly C (150 µg/mouse IP) 1 day previously or mice that had been immunized 1 week previously with 10^7 irradiated C1498 injected SC. Some mice received injection of a depletionary dose of NK1.1 antibody before harvesting splenocytes. (B) Draining LN cells were harvested from mice depleted of NK cells and immunized with irradiated C1498, and stimulated in vitro with irradiated C1498. Cytolysis was measured against C1498 or FBL (1A). Data shown are representative of three experiments.

The specificity of CTL lysis is illustrated in Fig 1B in which FBL leukemia (also derived from a C57BL/6 mouse, H-2b) is included as a target and is not lysed. Similar data were obtained utilizing EL4, YAC, or P815 cell lines as targets, despite demonstration that all these targets are effectively lysed by cytolytic effectors in vitro (data not shown).

These data show that NK cells, as well as an NK negative cell population, presumably T cells, can recognize and lyse C1498 in vitro. Cytolysis by NK-depleted CTL is higher than that observed with activated NK cells and approximates lysis by LAK cells (data not shown). Importantly, CTL generation requires in vivo exposure to a relatively large number of irradiated C1498 cells, presumably to provide a source of antigen for T-cell priming.

In vitro CTL lysis of C1498 leukemia is dependent on CD8 and LFA-1 on effectors and MHC class I, ICAM-1, and ICAM-2 on C1498. To determine which recognition molecules expressed on the surface of the effector and target cells are responsible for facilitating C1498 cytolyis, blocking studies were performed with MoAbs directed at surface molecules on either effector cells or leukemia cells. When either CD4 or CD8 was blocked on CTL, only CD8 blockade had an inhibitory effect, whereas blocking class I on C1498 diminished CTL lysis (Fig 2A). When the role of cell adhesion pathways in CTL lysis of C1498 was investigated, it was found that blockade of LFA-1 on the CTL, but not the leukemia, had a dramatic inhibitory effect upon in vitro lysis (Fig 2B). When blockade of the ligands for LFA-1 on C1498, ICAM-1 and/or ICAM-2, was performed, both
IMMUNE RESPONSE TO MURINE MYELOID LEUKEMIA

3

40-

E

tions of the indicated MoAb and tested for cytolysis of three experiments.

LFA-1 on effectors and MHC class II effectors. Draining LN cells were harvested from mice immunized with irradiated C1498, restimulated in vitro with irradiated C1498. Either the LAK effectors or C1498 targets were incubated with saturating concentrations of the indicated MoAb and tested for cytolyis of C1498 targets as described in Materials and Methods. Data shown are representative of three experiments.

MoAbs individually had equivalent inhibitory effects, while the combination of the two MoAbs was additive. The combination of anti-ICAM-1 and anti-ICAM-2 MoAbs did not equal the degree of inhibition achieved with blocking LFA-1 on the CTL, suggesting that one or more additional ligands for LFA-1 are present on C1498. Almost identical data were obtained when LAK effectors were used, indicating that both CD8+ CTL and activated NK cells are dependent on LFA-1 recognition of ICAM-1 and ICAM-2 on this myeloid leukemia cell line.

In vivo survival after intravenous (IV) injection of C1498 leukemia. IV injection of C1498 leukemia into congenic host mice (C57BL/6-Ly5.2) leads to reproducible death from leukemia in a dose-dependent fashion. As seen in Fig 3, 10^4 and 10^5 cells injected per mouse are uniformly lethal, 10^6 cells are partially lethal, and 10^7 cells are nonlethal. The use of the congenic host mice allowed the use of MoAbs (anti-Ly5.1 and anti-Ly5.2) to detect the C1498 leukemia in host tissues. The BM, liver, and LN were the tissues most heavily involved after IV injection of C1498. Some mice also developed subcutaneous masses comprised mostly of leukemia cells. This pattern is consistent with the leukemic involvement seen clinically with myelomonocytic leukemias consisting of hepatosplenomegaly and lymphadenopathy, as well as the occurrence of subcutaneous masses (chloromas).37

In vivo immune surveillance of a low leukemia burden is dependent on NK cells and requires LFA-1:ICAM interaction. As both NK cells and CD8+ T cells can recognize and lyse C1498 in vitro, we conducted experiments to determine if either or both types of effectors were involved in mediating leukemia resistance. Because we had determined that 10^4 C1498 cells/mouse was only partially lethal (see Fig 3), we asked whether NK or T cells were playing a role in protecting the host from a low dose of C1498 leukemia. Mice received depletionary doses of either anti-CD4 and anti-CD8 MoAb or anti-NK1.1 MoAb before IV injection of 10^4 C1498 leukemia cells, and then weekly antibody injections thereafter for 4 weeks. Another group received injections of anti-LFA-1 MoAb on a similar schedule.

Figure 4 represents cumulative data from two separate but reproducible experiments, and demonstrates that depletion of NK cells, but not T cells, has a statistically significant effect on survival. Thus, NK cells are able to eliminate a low dose (10^4) of C1498 leukemia. Furthermore, mice that

Fig 2. In vitro lysis of C1498 leukemia is dependent on CD8 and LFA-1 on effectors and MHC class I, ICAM-1, and ICAM-2 on C1498. Draining LN cells were harvested from mice immunized with irradiated C1498, restimulated in vitro with irradiated C1498. Either the effectors or C1498 targets were incubated with saturating concentrations of the indicated MoAb and tested for cytolyis of C1498 targets as described in Materials and Methods. Data shown are representative of three experiments.

Fig 3. Survival of mice injected IV with different doses of live C1498 leukemia cells. Congenic C57B1/6 Ly5.2 female mice were injected IV with the indicated doses of cells and followed for survival. Shown is Kaplan-Meier plot of survival where N = 19 to 22 (except 10^4 group N = 6). Data are pooled from two experiments.

Fig 4. In vivo immune surveillance of a low leukemia burden is dependent on NK cells, not T cells, and requires LFA-1. Mice received the designated MoAbs starting 3 days before IV injection of 10^4 C1498 and continued to receive MoAb injections for 4 weeks (N = 8 to 10/group for each of two experiments data pooled). P values are shown compared with control group.
received an in vivo blocking dose of anti-LFA-1 MoAb also had a significantly poorer survival, suggesting that the NK cell-mediated resistance was dependent on LFA-1 for recognition of C1498. The fact that T cells were not demonstrated to play a role in eliminating a low dose of C1498 was perhaps not surprising, given the fact that prior immunization with a higher number of irradiated leukemia cells was necessary to generate CTL in vitro.

SC or IP, but not IV, immunization with irradiated leukemia results in immune resistance to the systemic growth of C1498 cells that is associated with the expansion of C1498 leukemia reactive CTLp. T cells did not appear to participate in eradication of a 10^4 dose of C1498 cells, suggesting that the growth of the leukemia in vivo did not lead to sufficient priming and expansion of the T cells. We, therefore, asked whether or not leukemia reactive CTLp are expanded after growth of IV injected C1498. Frequency estimations of leukemia reactive CTLp were performed in naive mice, mice with systemically growing leukemia (but healthy in appearance), or mice immunized SC with irradiated leukemia. Mice that had received live leukemia cells had a less than twofold increase (f = 1:20, 611, 95% CI 1:14, 114-29, 795) in the frequency of leukemia reactive CTLp as compared with naive mice (f = 1:38, 239, 95% CI 1:26, 582-55, 009). In contrast, mice immunized with irradiated C1498 cells had a 28-fold increase in CTLp (f = 1:1.378, 95% CI 1:1060-1792) as compared with naive mice.

One possible explanation for these results is that the IV route of exposure to the leukemia was ineffective in stimulating CD8+ T cells. To test the hypothesis that the route of exposure determines the degree of expansion of CTLp, mice were immunized at weekly intervals by the IV, IP, or SC route with 10^5 irradiated C1498 cells. Two weeks after the second immunization, splenocytes were harvested for CTLp frequency analysis and the remaining mice challenged with 10^6 live C1498. Figure 5 shows that both IV and SC immunization effectively increased CTLp and provided protection against a lethal challenge of C1498. However, IV immunization had only a slight effect on either CTLp frequency or survival. These results show that the IV route of exposure of C1498 leukemia to the host immune system is not effective at generating a T-cell immune response.

SC immunization of naive mice with irradiated C1498 cells is more effective than exogenous IL-2 in treatment of leukemia-bearing mice. To investigate the possibility that the lack of a helper T-cell response and, therefore, a lack of IL-2 for expansion of CTLp could explain the failure of systemic growth of leukemia to stimulate an immune response, liposomal encapsulated IL-2 (25,000 IU/d) was administered SC daily for a period of 2 weeks starting on day 1 after a 10^5 dose of C1498. This dose of liposomal IL-2 has previously been shown to result in sustained levels of biologically active IL-2 in the serum for 24 hours in the range of 2 to 8 IU/mL, a level that should be adequate for T-cell stimulation. For comparison, another group received SC immunization with 10^5 irradiated C1498 on days 1 and 8. CTLp frequency was measured on day 8 for all groups.

As seen in Fig 6, the group receiving immunizations had a significantly higher actuarial survival rate as compared with the group receiving exogenous IL-2 (P < .002). Furthermore, CTLp were expanded in the immunization group, but not in the IL-2 group, which had a CTLp frequency identical to the control group. Increasing the dose of liposomal IL-2 to 250,000 IU/d, or the use of nonliposomal IL-2 had no beneficial effect on survival of mice receiving 10^5 C1498 (data not shown).

Retroviral-mediated IL-2 gene expression by C1498 cells reduces tumorigenicity through augmentation of CD8+ T cells and allows expansion of precursor CTL. Because others had shown that tumor cells engineered to make IL-2 could bypass the need for helper T cells in developing a CD8+ cytolytic T-cell immune response, we next investigated the possibility that local provision of IL-2, in contrast...
to systemic administration of IL-2, would allow systemically growing C1498 to expand CTLp and generate systemic immunity. C1498 was transduced with the human IL-2 expression vector LIL-2SN as described in Materials and Methods. The highest producing line secreted 4 to 5 IU of IL-2 per 10^6 cells in 24 hours and was chosen for further study compared with a control line transduced with the neo-vector LN. Both the control LN and IL-2–transduced sublines had identical in vitro growth characteristics, in vitro susceptibility to CTL and NK lysis, and comparable cell adhesion molecule expression as compared with the parental line (data not shown).

Mice survived IV injection of 10^5 or 10^6 of the IL-2–producing C1498 cells (C1498/IL-2) better than the control transduced C1498 cells (C1498/LN) (Fig 7). To understand what lymphocyte subsets might be involved in mediating elimination of the IL-2–producing C1498 leukemia cells, we performed in vivo depletion of CD4+ or CD8+ T cells, as well as NK cells, and challenged mice with C1498/IL-2. As can be seen in Table 1, depletion of CD8+ T cells had the most significant effect on survival, essentially eliminating the survival advantage of the mice receiving the IL-2 transduced leukemia versus the control transduced leukemia. Depletion of CD4+ T cells also had a significant effect on survival, which was less dramatic than CD8+ T cells, whereas depletion of NK cells did not significantly affect survival of mice receiving C1498/IL-2. Coinjection of live C1498/IL-2 with C1498/LN improved survival compared with mice receiving only C1498/LN, suggesting the generation of systemic immunity by the IL-2–producing C1498 (Fig 8). It is important to point out that the group receiving both C1498/LN and C1498/IL-2 were injected with 2 × 10^6 cells compared with all other groups that received only 10^5 cells. Therefore, the increase in survival rate for the coinjection group demonstrates the ability of localized IL-2 provision to stimulate an immune response, which can eliminate leukemia cells that are not producing IL-2. Further evidence for the generation of a systemic immune response as the result of local IL-2 provision by C1498 leukemia cells was obtained by measurement of CTLp frequencies on day 35 in the same experiment. Mice receiving 10^6 C1498/IL-2 had a substantially higher frequency of leukemia reactive CTLp (f = 1:66, 95% CI 1:45, 9:829) than mice receiving 10^5 C1498/LN (f = 1:35, 95% CI 1:22, 8:73–5:019), suggesting that the local provision of IL-2 augmented expansion of leukemia reactive CTL.

Table 1. Effect of IL-2 Gene Expression by C1498 Leukemia on In Vivo Survival and Role of Lymphocyte Subsets in Mediating In Vivo Survival Advantage

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Cell Dose</th>
<th>No.</th>
<th>MoAb Depletion</th>
<th>MST</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1498/IL-2</td>
<td>10⁴</td>
<td>9</td>
<td>—</td>
<td>34.4</td>
<td>—</td>
</tr>
<tr>
<td>C1498/LN</td>
<td>10⁵</td>
<td>9</td>
<td>—</td>
<td>31.8</td>
<td>.097</td>
</tr>
<tr>
<td>C1498/LN</td>
<td>10⁵</td>
<td>10</td>
<td>—</td>
<td>26.4</td>
<td>.00061</td>
</tr>
<tr>
<td>C1498/IL-2</td>
<td>10⁵</td>
<td>9</td>
<td>CD8</td>
<td>27.7</td>
<td>.0016</td>
</tr>
<tr>
<td>C1498/IL-2</td>
<td>10⁵</td>
<td>9</td>
<td>CD4</td>
<td>30.5</td>
<td>.025</td>
</tr>
<tr>
<td>C1498/IL-2</td>
<td>10⁵</td>
<td>9</td>
<td>NK1.1</td>
<td>32.1</td>
<td>.084</td>
</tr>
</tbody>
</table>

Mice were injected IV with the indicated cell line at the indicated cell dose on day 0. Groups receiving MoAb depletion were given the first dose of MoAb on day −3 and weekly thereafter per Materials and Methods.

Abbreviation: MST, median survival time.
* P value based on log-rank statistic compared with C1498/IL-2 10⁵ no MoAb depletion group.

DISCUSSION

The data presented in this report show that both NK cells and CD8+ CTL can recognize and lyse C1498 leukemia in vitro (Figs 1A and 2A). Both NK- and CTL-mediated killing of C1498 are dependent on LFA-1 binding to ICAM-1 and ICAM-2 present on C1498 (Fig 2B). With regard to in vivo immune surveillance mechanisms, host NK cells appear to be capable of elimination of a low number (10⁵) of C1498 cells injected IV, which is also dependent on LFA-1 binding (Fig 4). NK cell-mediated immune surveillance is apparently overwhelmed when a larger leukemia burden is injected because 10⁶ C1498 cells are uniformly lethal to an immunocompetent host (Fig 3).
T cells, on the other hand, could not be shown to play a significant role in the immune surveillance of a low dose \((10^5)\) of C1498, when injected into a naive host. However, mice immunized by the SC or IP (but not IV) route could then resist even a \(10^7\) dose of C1498 (Figs 5 and 6). This correlated closely with expansion of leukemia reactive CTLp, suggesting that T cells were then able to mediate immune surveillance of a relatively high leukemia burden. There are many possible reasons that IV immunization with C1498 failed to induce an effective T-cell immune response. Other investigators have shown that IV administration of an immunogen, in contrast to SC administration, fails to induce a T-cell immune response. One hypothesis proposed by these investigators is that when the immunogen is injected IV, antigen presentation by nonactivated APCs takes place with the result that T cells undergo an initial stimulation, but because less IL-2 is made, these T cells die from lack of growth factor stimulation. We reasoned, as well, that defective antigen presentation with a resultant lack of T cell help in the form of IL-2 could explain why IV immunization with C1498 was ineffective and could also possibly explain how C1498 leukemia cells escape T-cell immune surveillance after IV injection of live leukemia cells.

To test the hypothesis that a lack of T cell help in the form of IL-2 could explain failure of a T-cell immune response after IV injection of C1498, the leukemia cells were genetically engineered to produce IL-2. The IL-2–producing leukemia cells were more readily rejected than control transduced leukemia cells when live cells were injected IV (Fig 7), and this in vivo effect could be accounted for by T cells, primarily by CD8+ T cells (Table 1). Furthermore, IL-2 production by IV-injected C1498 leukemia cells facilitated leukemia reactive CTLp expansion and generation of a systemic immune response capable of eliminating non–IL-2 producing leukemia cells (Fig 8). Taken together, these data suggest that C1498 leukemia escapes immune surveillance by CD8+ T cells by failing to stimulate host expansion of leukemia reactive CTLp, which can be overcome by immunization by the proper route or local provision of IL-2 by the leukemia cells themselves. We speculate that the lack of expansion of leukemia reactive CTLp when C1498 grows in the host is due to lack of participation of professional APCs and, therefore, lack of a helper T-cell response with provision of helper cytokines, especially IL-2, necessary for expansion of CTLp.

When irradiated leukemia cells are injected SC (or IP), leukemia associated antigen(s) are presumably concentrated at a site of professional APC residence. Langerhans cells in the skin, for example, are known to process antigens, migrate to draining lymph nodes, and differentiate into dendritic cells, which then present antigen to T cells.\(^3\) Dendritic cells, which can then generate a clonal line of CD8+ CTL, which specifically lyse C1498 in vitro, is dependent on exogenous IL-2 for propagation, and secretes interferon-\(\gamma\) (data not shown). This is the typical cytokine profile of helper dependent CD8+ CTL.\(^3\) The absence of a murine retrovirus in the pathogenesis of the leukemia allows the study of T-cell responses to putative tumor antigens and not retroviral antigens.\(^3\) This CTL clone may prove useful in the elucidation of the putative C1498 tumor antigen(s), as has been accomplished by others.\(^3\)

In addition to recognition of antigen presented by MHC class I on the leukemia, recognition of C1498 by CD8+ CTL was completely dependent on LFA-1 binding to ICAM-1, ICAM-2, and perhaps other LFA-1 ligand(s) on C1498 (Fig 2B). The possibility that other LFA-1 ligand(s) are present on C1498 is interesting because ICAM-3 has recently been cloned in humans and found to be a LFA-1 ligand that is highly expressed by monocytes.\(^28\) Because C1498 has monocytic characteristics, it would not be surprising if it expressed a mouse homologue of human ICAM-3. ICAM-3 has not yet been cloned in mice and no antibody currently exists, so we cannot at present address this issue. To what extent human AML expresses ICAM molecules has not been thoroughly investigated. One report found that 85% of CD34+ AML blasts expressed ICAM-1, and blockade of LFA-1 on LAK cells significantly inhibited their ability to kill AML.
blasts. This suggests that LFA/ICAM interaction is likely to be important for effector cell recognition and lysis of human AML, as it is for murine AML in our model.

There are several possible implications for human AML, which could be derived from our study. For example, even if a given leukemia is very immunogenic, as is the case for C1498, a coordinated T-cell host immune response is not likely to take place. This is because leukemia growing systemically in the host does not appear to result in expansion of leukemia reactive CTL, which would appear to be due at least in part to lack of sufficient helper cytokines. Even if the particular myeloid leukemia expresses MHC class II, as the majority of human AML do (with the exception of French-American-British type M3), allowing antigen presentation to CD4+ T helper cells, they will likely lack critical costimulatory molecules, such as B7-1 or B7-2. Direct antigen presentation by the leukemia cells in the absence of sufficient costimulation would be expected to result in anergy (antigen specific hyporesponsiveness) induction of CD4+ T helper cells, as has been shown in human melanoma. Thus, we postulate that without adequate antigen presentation by professional APCs with consequent stimulation of a helper T-cell response, or the local provision of helper cytokines to bypass the need for T helper cells, CD8+ T-cell-mediated immune surveillance of leukemia is not able to take place.

Current clinical strategies for immunotherapy of AML are focused on exogenous provision of high-dose IL-2. Preliminary data with the use of high-dose IL-2 in conjunction with autologous bone marrow transplant (BMT) appears promising. Indeed, others have used the C1498 cell line in a model of autologous BMT with exogenous IL-2 and adoptive immunotherapy and demonstrate a therapeutic effect. These responses seen clinically and in the preclinical model are very likely caused by the generation by high-dose IL-2 of non-MHC-restricted LAK cells. However, based on in vitro data, it seems unlikely that all cases of AML will be susceptible to LAK-based immunotherapy. Furthermore, in addition to in vivo toxicity associated with IL-2, in vivo generation of LAK effectors is short-lived, in contrast to the long-term memory response characteristic of T cells. For these reasons, we believe that T-cell-mediated approaches to immunotherapy of AML also warrant investigation.

Successful immunotherapy of AML by leukemia specific CD8+ CTL should, therefore, be focused on either one of two different strategies. One strategy would be to optimize adequate antigen presentation of leukemia antigens by professional APCs to both CD4+ and CD8+ T cells. This would be predicted to be successful only if leukemia antigens that can be presented by both MHC class I and class II exist. Recently, it has been demonstrated that granulocyte-macrophage colony-stimulating factor (GM-CSF) secretion by transduced murine tumors induced potent CD4+ and CD8+ antitumor immunity. This is presumably due to the ability of GM-CSF to serve as a major growth factor for dendritic cells. Another possibility would be to load leukemia antigens directly into professional APCs and then to use these in vivo as a vaccine. A better understanding of what constitutes leukemia-associated antigens would likely be necessary to accomplish this strategy.

The alternative strategy is to deliver IL-2 locally to CD8+ CTL that have had recent leukemia antigen presentation by MHC class I molecules. This strategy would likely be necessary if the leukemia only contained antigen(s) capable of being presented by MHC class I, but not MHC class II, molecules. The fact that we were unable to generate an in vivo antileukemia CTL response by exogenous IL-2 (Fig 6) suggests that further studies are necessary to understand if it is possible to deliver IL-2 exogenously to leukemia specific CD8+ CTL in vivo. IL-2 gene transfer into leukemia cells appears to represent a physiologic and effective method for bypassing the need for T helper cells in the generation of a leukemia specific CD8+ CTL response in the host.

REFERENCES

15. Gregory CD, Murray RJ, Edwards CF, Richardson AB: Down-regulation of cell adhesion molecules LFA-3 and ICAM-1 in Epstein-Barr virus-positive Burkitt's lymphoma underlies tumor cell

From www.bloodjournal.org by guest on November 16, 2017. For personal use only.
16. Glasebrook AL, Fitch FW: T cell lines which cooperate in
17. Brenner MK, Heslop HE: Immunotherapy of leukemia. Leu-
kemia 6:76, 1992 (suppl 1)
18. Cepko C: Reverse transcriptase assay to detect helper virus,
in Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG,
Smith JA, Struhl K (eds): Current Protocols in Molecular Biology,
vol 1. Brooklyn, NY, Greene Publishing and Wiley-Interscience,
1993, p 13.3
CM, Goldstein M, Ochoa AC: Increased local antitumor effects of
interleukin-2 liposomes in mice with MCA-102 sarcoma pulmonary
20. Wunderlich J, Shearer G: Induction and measurement of cyto-
toxic T lymphocyte activity, in Coligan JE, Kruisbeek AM, Margu-
lies DH, Shevach EM, Strober W (eds): Current Protocols in Immu-
3.11.1
21. Taswell C: Limiting dilution assays for the determination
of immunocompetent cell frequencies: I. Data analysis. J Immunol
126:1614, 1981
22. Katsanis E, Orchard PJ, Bausero MA, Gorden KB, McIvor
RS, Blazar BR: Interleukin-2 gene transfer into murine neuro-
blastoma decreases tumorigenicity and enhances systemic immunity
cauing regression of preestablished retropertitoneal tumors. J Immun-
other 15:81, 1994
23. Kaplan EL, Meier P: Nonparametric estimation from incom-
24. Peto R, Peto J: Asymptotically efficient rank invariant proce-
25. Dunham LJ, Stewart HL: A survey of transplantable and
SF, Hasz D, Ochoa AC, Brostrom D: Depot characteristics and biodistribution of interleukin-
2 liposomes: Importance of route of administration. J Immunother
12:19, 1992
27. Feorun ER, Pardoll DM, Itaya T, Golubeck P, Levitsky HI,
Simons JW, Karasuyama H, Vogelstein B, Frost P: Interleukin-2 production by tumor cells bypasses T helper function in the genera-
28. Keanney ER, Pape KA, Loh DY, Jenkins MK: Visualization of peptide-specific T cell immunity and peripheral tolerance induc-
tion in vivo. Immunity 1:327, 1994
29. Steinman RM: The dendritic cell system and its role in immu-
30. Unanue ER: Macrophages, antigen-presenting cells, and the
phenomena of antigen handling and presentation, in Paul WE (ed):
111
31. Huang AY, Golubeck P, Ahmadzadeh M, Jaffee E, Pardoll
D, Levitsky H: Role of bone marrow-derived cells in presenting
33. Feng TA, Mosmann TR: Alloreactive murine CD8 T cell
clones secrete the Th1 pattern of cytokines. J Immunol 144:1744,
1990
34. Holf CA, Osorio K, Lilly F: Friend virus-specific cytotoxic
T lymphocytes recognize both gag and env gene-encoded specificities.
35. Boon T, Cerottini JC, Van den Eynde B, van der Bruggen P,
Van Pel A: Tumor antigens recognized by T lymphocytes. Annu
Rev Immunol 12:337, 1994
36. de Fougerolles AR, Qin X, Springer TA: Characterization of the function of intercellular adhesion molecule (ICAM)-3 and
comparison with ICAM-1 and ICAM-2 in immune responses. J Exp
Med 179:619, 1994
37. Henderson ES, Afshani E: Clinical manifestation and diagno-
sis, in Henderson ES, Lister TA: Leukemia (5th ed), Philadelphia,
PA, Saunders, 1990, p 291
38. Becker JC, Brabletz T, Czerny C, Termeer C, Brocker EB:
Tumor escape mechanisms from immunosurveillance: Induction of
unresponsiveness in a specific MHC-restricted CD4 human T cell
cline by the autologous MHC class II+ melanoma. Int Immunol
5:1501, 1993
39. Fife A, Benyunes MC, Massumoto C, Higuchi C, York A,
Buckner CD, Thompson JA: Interleukin-2 therapy after autologous
bone marrow transplantation for hematologic malignancies. Sem
Oncol 20:41, 1993 (suppl 9)
40. Charak BK, Brynes RK, Groschen S, Chen S, Mazumder A:
Bone marrow transplantation with interleukin-2 activated bone mar-
row followed by interleukin-2 therapy for acute myeloid leukemia in
41. Dranoff G, Jaffee E, Lazebny A, Golubeck P, Levitsky H,
Brose K, Jackson V, Hamada H, Pardoll D, Mulligan RC: Vaccin-
cination with irradiated tumor cells engineered to secrete murine granulo-
cyte-macrophage colony-stimulating factor stimulates potent, specif-
ic, and long-lasting anti-tumor immunity. Proc Natl Acad Sci USA
90:3539, 1993
42. Steinman RM: Granulocyte/macrophage colony-stimulating factor is essential for the viability and function of cultured murine epidermal
43. Cohen PJ, Cohen PA, Rosenberg SA, Katz SI, Mule JI: Mu-
rine epidermal Langerhans cells and splenic dendritic cells present
tumor-associated antigens to primed T cells. Eur J Immunol 24:315,
1994
Dependency on intercellular adhesion molecule recognition and local interleukin-2 provision in generation of an in vivo CD8+ T-cell immune response to murine myeloid leukemia

MW Boyer, PJ Orchard, KB Gorden, PM Anderson, RS Mclvor and BR Blazar