Cell-mediated Immunity to Leukemia Virus- and Tumor-associated Antigens in Mice

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Summary

Cell-mediated immune reactions appear to play an important role in resistance against growth of leukemia cells in mice. Possible mechanisms for in vivo protection in two tumor systems are discussed. These tumor models, which are a Friend leukemia virus-induced transplantable tumor, FBL-3, and primary murine sarcoma virus (MSV)-induced tumors, are strongly antigenic; under some conditions, tumors regress completely. In mice with regressing FBL-3 tumors, cell-mediated cytolysis was measured by release of $^{31}$P-iododeoxyuridine. The response was biphasic, with an initial peak at 10 days and a 2nd peak after 30 days. A boost in reactivity could be elicited by later challenge with tumor cells. All of the reactivity was dependent on T-cells, being eliminated by treatment with anti-Ig plus complement. The specificity of the reactions was not completely defined, but it was consistent with Friend type-specific antigen plus broader, common antigens. In mice with regressing MSV tumors, strong cell-mediated cytolysis, measured mainly by release of $^{51}$Cr, was seen against RBL-5, a Rauscher virus-induced leukemia. A single peak of response occurred at about 14 days after virus inoculation. Upon later challenge with RBL-5 cells, a vigorous and rapid secondary response was elicited, mainly in the region of tumor challenge. This cytotoxicity also was completely dependent on T-cells. In addition, macrophage-mediated inhibition of leukemia cell growth in vitro was seen in this system at the time of peak tumor development. The $^{51}$Cr release cytotoxicity was specific and directed primarily against an antigen, MEV-SA1, associated with mouse endogenous C-type viruses. The macrophage-induced growth inhibition appeared to be nonspecific. In both the FBL-3 and MSV tumor systems, protection against tumor growth could be adoptively transferred by immune lymphoid cells. In addition to induction of cell-mediated immunity by tumor cell or virus inoculation, cell-mediated cytotoxic reactivity was found to occur naturally in most young mice. This natural killer activity was quite distinct from the experimentally elicited reactions, being mediated by N-cells, a subpopulation of lymphoid cells with no clearly identifiable cell surface markers. The natural cytolysis was also directed against antigenic specificities different from those recognized by the MSV-immune cells. The central issue in all of these studies has been to determine the relationships between the in vitro-detected cell-mediated reactivity and in vivo resistance to leukemia.

Introduction

There have been many recent in vitro studies of the cell-mediated immune response to MSV-induced tumors and to various syngeneic leukemias in mice. We will not attempt to review all of these studies here, but we will focus on several important issues and present some data to illustrate these points. The central problem is to determine the effector mechanisms responsible for resistance of the host against tumor growth. Mouse leukemia cells contain several different tumor-associated cell surface antigens. The tumor-associated cell surface antigens on leukemia cells may be leukemia-associated antigens, antigens associated with the inducing or with endogenous C-type viruses, or fetal antigens; 1 or more of these antigens may be detected by assays for cell-mediated immunity. It is important to ascertain which of these antigens can function as TATA. In addition to the complexities of specificity, different lymphoid cell populations may mediate cellular reactions against leukemia cells. Recently, it has also become apparent that cells from normal mice, as well as from deliberately immunized mice, may have cell-mediated reactivity against leukemia cells. We shall discuss some approaches to the analysis of the specificity and mechanisms of cell-mediated immune reactions to mouse leukemia cells and try to relate this information to in vivo protection against tumor growth. We will also present some recent information on augmentation of the cell-mediated cytotoxic response to leukemia cells, which may be very relevant to considerations of immunological control of tumors.

Friend Virus-induced Leukemia, FBL-3, in C57BL/6 Mice

This transplantable leukemia system has been of particular interest because it is antigenic and it has been possible to induce tumors that grow transiently and then regress, and also tumors that grow progressively and kill the host (19). This has allowed us to contrast the immunological
responses associated with these divergent patterns of tumor growth. Inoculation of $5 \times 10^6$ FBL-3 cells s.c. into syngeneic recipients produced transient tumor growth (designated regressors); the tumor reached peak size at 20 to 30 days and then regressed. In contrast, i.p. inoculation of as few as $10^6$ cells produced progressor tumors and death. A cell-mediated immune response to FBL-3 could be measured by $[^{38}S]$IUdR release cytotoxicity assay (7, 20). In regressor mice, the kinetics of the cytotoxic reactivity was biphasic, with initial high reactivity at 10 days, decline to undetectable levels at 20 to 25 days, and reappearance of activity which then persisted for at least 60 days. In progressor mice, much lower activity was seen at any time point. All of the cell-mediated cytotoxic reactivity in this system, both during the early and late phases of response, was dependent on T-cells. Reactivity could be completely eliminated by treatment with anti-$\theta$ antibodies plus complement.

The levels of cell-mediated cytotoxicity were found to be dependent on the dose of inoculated tumor cells (Table 1), with higher responses elicited by larger numbers of cells. The higher reactivity could be achieved either by increasing the dose of a single inoculation or by giving repeated weekly inoculations of smaller cell doses. However, once a peak primary response of 20 to 30% cytotoxicity was achieved, higher doses or more inoculations over a short period had no additional effect. In addition to augmenting the height of the primary response, it has been possible to elicit a secondary cytotoxic response (Table 2). Previous inoculation of viable FBL-3 cells s.c. conferred complete protection against i.p. challenge with FBL-3. Within three days after challenge, highly cytotoxic lymphocytes could be detected in the peritoneal cavity and in the spleen.

The in vivo resistance to growth of FBL-3 appeared to be related to 2 antigenic specificities, a Friend type-specific transplantation antigen and a FMR common transplantation antigen (20). After primary immunization, most of the cytotoxic response was directed against FBL-3 (Table 2), with weak reactivity against RBL-5, a Rauscher virus-induced leukemia, and also against a subline of EL-4. After secondary immunization, the reactivity to FBL-3 and to RBL-5 appeared to be increased. It might be concluded from these direct cytotoxicity results that the primary response was mainly directed against a Friend type-specific antigen, with only a weak response against FMR antigen, and that the secondary response was fairly vigorous against both of these antigens. The weak reactivity in the primary and secondary response against EL-4(G+) might be ascribed to either the serologically defined Gross cell surface antigen or to MEV-SA1, a cell surface antigen associated with mouse endogenous C-type viruses (Ref. 3; see also next section). However, as will be discussed below, the data from direct cytotoxicity against various lines can provide only some indications of the antigenic specificities involved. More extensive testing, particularly with an inhibition assay, will be needed to define clearly the nature of the antigens detected in the primary and secondary responses.

In order directly to relate the in vitro cytotoxic reactivity of lymphoid cells from immunized mice to in vivo resistance to tumor growth, adoptive transfer experiments have been performed. Cells from regressor and progressor mice were tested for the in vitro reactivity and also for their ability to protect against i.p. challenge with FBL-3 cells (Table 3). The cells from regressors, which had considerable in vitro reactivity, gave complete protection, whereas those from progressors gave only partial protection.

### Table 1

**Development of cell-mediated cytotoxicity against FBL-3 after single and multiple inoculations of viable tumor cells**

<table>
<thead>
<tr>
<th>Dose of tumor cells</th>
<th>No. of doses</th>
<th>% cytotoxicity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>p-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1 \times 10^5$</td>
<td>1</td>
<td>12.7</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>$1 \times 10^6$</td>
<td>4</td>
<td>21.2</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>$5 \times 10^5$</td>
<td>1</td>
<td>18.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$5 \times 10^8$</td>
<td>4</td>
<td>24.4</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

<sup>a</sup> C57BL/6 mice inoculated s.c. with viable FBL-3 ascites tumor cells, either once or at weekly intervals. Tests performed 10 days after last inoculation.

<sup>b</sup> Percentage of cytotoxicity above normal control, measured by $[^{38}S]$IUdR release assay, against FBL-3 tissue culture cells, with ratio of spleen cells:tumor cells of 1000:1 (21).

### Table 2

**Cytotoxic reactivity of lymphocytes from C57BL/6 mice against various target cells after primary and secondary immunization with viable FBL-3 ascites tumor cells**

<table>
<thead>
<tr>
<th></th>
<th>FBL-3</th>
<th>RBL-5</th>
<th>EL-4(G+)</th>
<th>EL-4(G-)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunization&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.8</td>
<td>7.7</td>
<td>6.5</td>
<td>ND&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Secondary</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunization&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.0</td>
<td>16.5</td>
<td>6.2</td>
<td>-1.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Percentage of cytotoxicity above normal control, measured by $[^{38}S]$IUdR release assay, against target cells growing in tissue culture, with attacker:target cell ratio of 1000:1 for primary immunization and 500:1 for secondary immunization.

<sup>b</sup> C57BL/6 mice inoculated s.c. with $5 \times 10^6$ viable FBL-3 ascites tumor cells, and lymph node cells tested after 10 days.

<sup>c</sup> ND, not done.

<sup>d</sup> Forty days after primary immunization, C57BL/6 mice were inoculated i.p. with $1 \times 10^5$ viable FBL-3 cells, and spleen cells were tested 6 days later.
TATA's on virus-induced leukemias, it is a very useful model for analyzing cellular immunity to leukemia. In contrast to MSV results in transient local tumor growth (16), the tumor gists. This system, like the FBL-3 system, is a very antigenic one, and it is possible to produce regressing tumors as well as progressing tumors. Inoculation i.m. of most stocks of MSV even in adult recipients.

The induction of cell-mediated immunity by MSV has been a subject of intense interest to many tumor immunologists. This system, like the FBL-3 system, is a very antigenic one, and it is possible to produce regressing tumors as well as progressing tumors. Inoculation i.m. of most stocks of MSV results in transient local tumor growth (16), the tumor reaches maximal tumor growth at about 10 days and then rapidly and completely regresses. Thereafter, the mice are strongly resistant to challenge with virus or with leukemias induced by Friend, Rauscher, or Moloney virus. Because MSV inoculation can produce strong immunization of TATA's on virus-induced leukemias, it is a very useful model for analyzing cellular immunity to leukemia. In contrast to the usual regression of MSV tumors, progressive tumor growth can be induced by regular stocks of MSV in very young or in immunosuppressed mice or by some stocks of MSV even in adult recipients.

In parallel with the common TATA's between MSV and virus-induced leukemias, demonstrated by in vivo protection, mice inoculated with MSV also show strong in vitro cytotoxicity against some leukemias. We have studied this with a 4-hr \(^{51}Cr\) release assay, using the Rauscher virus-induced leukemia RBL-5 as the target cell (16). In regressors, cytotoxicity reached a peak at about 14 days and then rapidly declined to low levels. Progressors had lower levels of reactivity, but the kinetics of response was very similar to those seen with regressors.

The kinetic pattern of reactivity seen in the \(^{51}Cr\) release assay was quite different from the biphasic response observed by other investigators using the visual microcytotoxicity assay (15, 18). The kinetics of reactivity that we induced by injection of FBL-3 and measured by the long-term \(^{3}H\)UdR release assay seemed much more similar to those described for the microcytotoxicity assay. It was possible that, similar to the comparison between the \(^{51}Cr\) release assay and the microcytotoxicity assay in a rat leukemia system (23), many of the differences could be accounted for by the different incubation periods in the assays. It was, therefore, of interest directly to compare different assays for cell-mediated cytotoxicity in the same laboratory, using MSV inoculation as the common means of immunization. Cytotoxicity was measured in the visual microcytotoxicity assay and in the \(^{3}H\)UdR release assay, both with 40-hr incubation periods, and in the 4-hr \(^{51}Cr\) release assay (1).

With the particular target cells used for this study, virtually the same kinetics of response was seen in all three assays, and there was not even a suggestion of a biphasic response in the long term assays.

In the \(^{51}Cr\) release assay, we have performed extensive studies of the specificity of the reactions by testing for inhibition with unlabeled target cells (3). Because of the reactivity of MSV-immune cells against leukemias like RBL-5, it seemed likely, and indeed other groups have assumed (e.g., Ref. 17), that the antigens were related to the serologically defined FMR antigens. However, we have found that the main antigenic specificity recognized by the cytotoxic cells is distinct from any of the expected specificities. Transplantable BALB/c leukemias induced by Friend, Rauscher, or Moloney viruses, fetal cells, and most virus-induced tumors in other species lacked detectable antigen. The antigen appears to be related to expression of mouse endogenous C-type virus and has been designated MEV-SA1 (3). Many mouse tumor lines, in transplant or in tissue culture, of various etiologies have been found to have expression of endogenous C-type viruses and to contain MEV-SA1 (3). Preliminary studies have indicated that the endogenous virus-associated antigens can function as TATA's, since immunization with MSV produced protection against EL-4(G+), which contains MEV-SA1, and not against EL-4(G-), another subline of the same tumor which lacks detectable amounts of MEV-SA1.

It had initially appeared, in our own studies and in those of others (17), that after regression of MSV tumors only low levels of spleen cell cytotoxicity could be detected and that there was no rise in reactivity after secondary challenge with leukemia cells. This cast some doubt on the in vivo relevance of the \(^{51}Cr\) release assay, since the mice were strongly protected against growth of the leukemia cells. Recently, however, we have found that a secondary challenge elicits a rapid and vigorous cell-mediated cytotoxic response that is often confined to the region of challenge (9). Table 4 shows the results of an experiment in which MSV-immune mice were challenged with RBL-5 ascites tumors cells. At 5 weeks after primary immunization, low levels of reactivity were found in PE cells and in inguinal lymph node cells. After i.p. challenge, very high cytotoxicity of PE cells was found after 2 days. After i.m. challenge, the draining lymph node cells developed a transient increase in reactivity on Day 6, whereas spleen cells only showed increased activity on Day 3. When mice were challenged at 8 months after primary immunization with MSV, the response

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of PE cells and of draining lymph node cells took longer to appear but still reached very high levels. No increased activity was seen with spleen cells after i.m. inoculation. The ability of immune mice to develop a rapid and strong cytotoxic response in the region of tumor challenge may be a critical element in the resistance to tumor growth. The specificity of the secondary response appears similar to that of the primary response, involving mainly MEV-SA1. However, some reactivity also appears to be directed against FMR antigens.

As in the FBL-3 tumor system, both the primary (6) and the secondary (9) cytotoxic response, measured in the ^51Cr release assay, is dependent upon and appears to be mediated by T-cells. Treatment of effector cells with anti-TH antibodies plus complement removed most of the cytotoxic reactivity.

In addition to the specific T-cell-mediated cytotoxic response after MSV inoculation, we have also observed the development, at about the same time, of nonspecifically reactive macrophages (13, 14). These cells do not react in the ^51Cr cytotoxicity assay but produce potent inhibition of in vitro proliferation, as measured by ^3Hthymidine incorporation. A variety of leukemia cells, with or without MEV-SA1, were susceptible to growth inhibition by these cells (Table 5). The evidence for mediation of these effects by macrophages is the resistance to treatment with anti-TH antibodies plus complement removed most of the cytotoxic reactivity.

As in the FBL-3 tumor system, we have initiated adoptive transfer experiments to correlate the in vivo resistance to tumor growth. Cells from mice immunized 13 to 40 days previously with a regressor stock of MSV gave almost complete protection against challenge of recipients with MSV (8). Lymphoid cells from regressor mice, at the peak of the cytotoxic response, prevented tumor appearance. Adoptive transfer of MSV-immune cells could also protect against challenge with RBL-5 leukemia cells (Table 6). Cells from regressors gave some protection when taken at 15 or 21 days. From regressors, the Day 15 cells did not protect but the Day 21 cells were effective. Therefore, despite the fact that the regressor donors would have died with MSV tumors, their lymphoid cells had some ability to protect other mice against challenge with MSV or RBL-5.
Natural Cell-mediated Immunity against Mouse Leukemias

In addition to induction of immunity to mouse leukemias and murine C-type viruses, there is increasing evidence for natural immunity to antigens associated with these tumors and viruses. Many normal mice have been shown to have antibodies against cell surface antigens associated with C-type viruses (see evidence presented by Dr. Hanna elsewhere in this Symposium). We and others have also found considerable evidence for natural cell-mediated immunity against mouse leukemias (5, 6, 8, 12). Some mice of many different inbred strains and also random-bred mice were found to have considerable cytotoxicity against RBL-5 and other mouse leukemias in the 4-hr 51Cr release assay. Table 7 summarizes some of those results. CBA mice had particularly high reactivity, whereas A mice had virtually none. Germ-free, as well as conventionally reared, BALB/c mice had intermediate levels of reactivity that were not appreciably different from each other. A surprising observation, in view of the T-cell dependence of the experimentally induced immune reactivity in this assay, was the consistently high cytotoxicity of nude, athymic mice. In all of the strains tested, reactivity was maximal in young mice, usually between 5 and 8 weeks of age. At ages over 12 weeks, when deliberately immunized mice were usually tested, only low levels of natural reactivity were detected.

The natural cell-mediated cytotoxicity was found to have specificity, but with a pattern distinct from that seen with cells from mice immunized by MSV or FBL-3. Leukemia cells of different types and from different strains, including YAC, an A-strain tumor induced by Moloney leukemia virus, and RL-δ1, a BALB/c tumor induced by radiation, were very susceptible to lysis. Although cells with high cytotoxic reactivity had similar effect on all of these cells, analysis of specificity by inhibition assays showed a multiplicity of antigens (Table 8). RL-δ1 and YAC contained antigens not found on RBL-5 cells, and each of these 2 cell lines contained antigens not present on the other. Since all of the antigen-positive cell lines have been found to contain mouse endogenous C-type viruses, it seems likely that all of the antigens are associated with these viruses. However, there were some striking differences in the specificity of natural reactivity and that of the reactivity induced by MSV. For example, the EL-4(G+) and EL-4(G−) sublines gave opposite patterns when tested for their ability to inhibit natural and MSV-immune lysis of RBL-5 target cells. Therefore, the antigens detected by the natural killer cells appear to be distinct from MEV-SA1 and have been tentatively designated MEV-SA2, 3, and 4 (5).

As suggested by the high cytotoxic reactivity of lymphoid cells from nude mice, the natural cytotoxic reactivity has been found to be independent of T-cells (4, 6, 11). Detailed studies to characterize the effector cells have failed to detect any characteristic marker (Table 9). In contrast to the effects on MSV-immune reactivity, treatment of nude spleen cells with anti-θ plus complement caused, at most, a partial reduction in cytotoxicity. Treatments affecting macrophage-mediated activity did not inhibit natural cytotoxicity. In fact, passage of cells over nylon columns, which can deplete macrophages and B-cells, gave a considerable increase in activity. Kiessling et al. (11) also failed to remove natural killer cells on columns containing antiimmunoglobulins. The natural cytotoxicity appears to be quite distinct from antibody-dependent cell-mediated cytotoxicity, since this type of reactivity in the mouse is mediated by adherent cells (21) with receptors for immunoglobulins (10) or for antibody-dependent cell-mediated cytotoxicity (22), during the cytotoxicity reaction had no inhibitory effect on natural

### Table 7

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Median % cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6N</td>
<td>4.2 (0.5–25)*</td>
</tr>
<tr>
<td>BALB/cN</td>
<td>4.1 (1.1–17.9)</td>
</tr>
<tr>
<td>BALB/c germ-free</td>
<td>1.4 ([−3.8]–12.5)</td>
</tr>
<tr>
<td>CBA/N</td>
<td>15.0 (12–22)</td>
</tr>
<tr>
<td>A/Jax</td>
<td>0.4 ([−0.1]–1.9)</td>
</tr>
<tr>
<td>BALB/c nude</td>
<td>15 (5.3–55)</td>
</tr>
</tbody>
</table>

*a Numbers in parentheses, range.

### Table 8

<table>
<thead>
<tr>
<th>Inhibitor cells</th>
<th>Strain of origin</th>
<th>BALB/c nude vs. various-labeled target cells</th>
<th>MSV immune vs. RBL-5 target cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBL-5 TC</td>
<td>C57BL/6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>YAC TC</td>
<td>A</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>RL-δ1 TC</td>
<td>BALB/c</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EL-4(G−) A</td>
<td>C57BL/6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MCDV-12 A</td>
<td>BALB/c</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>YC-8 A</td>
<td>BALB/c</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>EL-4(G+) A</td>
<td>C57BL/6</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

*a TC, tissue culture adapted tumor cells; A, ascites tumor cells; +, positive inhibition.
cytotoxicity. Because of the distinctness of the natural killer cells from any previously defined, and because the so-called "null cells" have been found to have receptors for immunoglobulins or for complement (2, 12), we have proposed to designate these cells tentatively as N-cells (4).

The relevance of this natural cytotoxicity against many mouse leukemia cells largely remains to be determined. Kiessling et al. (personal communication) have some evidence for correlation between levels of natural reactivity in a particular inbred strain and resistance to growth of transplantable leukemia cells. It will be important to determine the role of this mechanism in natural protection against leukemia.

### Discussion

As part of an approach to develop methods to control virus-associated tumors, it is very valuable to be able accurately to measure relevant cell-mediated immune reactions to TATA's. An understanding of the mechanisms of immunity and studies of how to augment this immunity may help to provide a rational means for preparing protocols for studies of in vivo protection or control of tumors. This approach may be more satisfactory than the more usual one of intuitively determining the details of treatment regimens and performing empirical studies, with only the difficult criteria of lack of detectable tumor growth or of survival as the end points for evaluation of efficacy. It is clear from studies such as those presented here that the cellular immune response is very complex, and we must be very cautious in directly relating data obtained in vitro to in vivo effector mechanisms. However, one can begin to understand the role of the various reactions by carefully studying the time course of the responses and the relation to the time course of tumor growth or regression, the specificity of the reactions and the relationship of the detected antigens to TATA's, and the nature of the effector cells in relation to the type of cells that are capable of inhibiting tumor growth in vivo. The present data on the localization of reactive cells in the secondary response also emphasize the point that studies of cell-mediated reactivity in 1 central organ, usually the spleen, may not be adequate to evaluate the response to tumors injected in a distant site. Recent studies in our laboratory have indicated that highly cytotoxic cells can even be isolated from within primary MSV tumors (H. T. Holden, S. Haskill, H. Kirchner, and R. B. Herberman, unpublished observations). Studies of immune cells in the region of the tumor or within the tumor itself may be much more relevant to the mechanism of protection against tumor growth than are studies of only spleen cell reactivity.

The natural cell-mediated immunity to leukemia, mediated by an unusual subpopulation of lymphoid cells, may be very important for in vivo surveillance or control of tumor growth. However, all of the work in this area has been done very recently and much remains to be studied. It may be particularly valuable to determine the mechanisms for development of this reactivity and the factors affecting the levels of activity. We have found, in preliminary studies, that inoculation of C57BL/6 mice with MSV causes a decrease in the levels of natural cytotoxic reactivity against RL-5 cells (5). In contrast, inoculation of irradiated RBL-5 cells into nude mice produced a considerable increase in cytotoxic reactivity (R. B. Herberman and H. T. Holden, unpublished observations). These findings indicate that exposure to antigens may have an influence on the levels of natural reactivity. However, germ-free or pathogen-free mice and mice from various backgrounds and environmental exposures had similar amounts of reactivity (5). The internal environment of the mice, and factors influencing activation of endogenous C-type viruses and their associated antigens, may play a more critical role. In addition, and perhaps more importantly, the genetic background of the mice and the presence or absence of thymic functions appear to affect the expression of natural cytotoxic reactivity. In any event, it should be possible to design experiments to determine the effect of different levels of natural cytotoxic reactivity on leukemia incidence and on protection against challenge with leukemia cells.

The results of studies of cell-mediated immunity to mouse leukemias which have been obtained to date are encouraging, in the sense that potential mechanisms for host protection against tumor growth have been identified and can now be readily studied. In fact, we now face the problem of having an excessive number of in vitro defined, potential
mechanisms. Which of these mechanisms play the predominant role \textit{in vivo}? Do the various types of effector cells have additive or synergistic antitumor properties, or do they actually interfere with each other \textit{in vitro}? These are some of the important questions that will hopefully be answered in the near future.

References


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*Cancer Res* 1976;36:615-621.