THE CLINICAL IMMUNOBIOLOGY OF INTERLEUKIN-2: POTENTIAL MODIFIED USES FOR IMPROVED CANCER TREATMENT*

STEPHAN D. VOSS, B.A., GILDA WEIL-HILLMAN, PH.D., JACQUELYN A. HANK, PH.D., JEFFREY A. SOSMAN, M.D., AND PAUL M. SONDEL, M.D.

Departments of Human Oncology, Pediatrics, and Genetics
University of Wisconsin-Madison
Madison, Wisconsin

RECENT technical developments have enabled the component elements of the immune system to be separated and analyzed individually. These have restimulated investigations into the possibility that mechanisms similar to those involved in protection from microbial pathogens might be utilized to allow selective destruction of tumor cells. A major contribution has been the discovery and purification of the primary active element of T-cell growth factor—Interleukin-2, allowing the in vitro and in vivo growth and expansion of a variety of immune cells.1,2 This review summarizes recent studies from our and other laboratories in which interleukin-2 plays an important role in the development of immunotherapeutic approaches to the treatment of cancer.

Preclinical Studies

IMMUNOGENIC TUMORS: ANTIGEN-SPECIFIC ANTITUMOR ACTIVITY

To evaluate the interactions between the immune system and neoplastic cells, experimentally induced murine tumors have been studied in vivo and in vitro. These tumors could be induced by oncogenic viruses, chemical carcinogens, or such physical agents as ultraviolet radiation. One hallmark of these experimentally induced tumors is their immunogenicity. Immunization of

*Presented as part of a Symposium on Combination Therapies: New and Emerging Uses for Cyclooxygenase Inhibitors, Calcium Blockers, and Biological Response Modifiers on Immunity, held by the Section on Medicine of the New York Academy of Medicine and the George Washington University School of Medicine and Health Sciences at the Essex House, New York, N.Y. June 2 and 3, 1988, and supported in part by an educational grant-in-aid from the Aspirin Foundation of America, Inc.

This work was supported by NIH contract CM-47669, NIH grants CA-32685, and RR-03186, and American Cancer Society grant CH-237.

S.D.V. is a Lutheran Brotherhood Scholar of the Life and Health Insurance Medical Research Fund.
syngeneic animals with nonviable tumor or tumor fragments renders them immune to subsequent challenge with viable tumor cells.\textsuperscript{3} Such immunogenic tumors characteristically express tumor-specific antigens on their surface, allowing them to be recognized by specific cytotoxic T cells bearing appropriately rearranged T cell receptor molecules (the α, β/CD3 complex). This typically requires the corecognition of autologous Class I major histocompatibility complex antigens also present on the surface of the tumor cells, the major histocompatibility complex antigens serving to "present" the tumor-specific antigens to autologous T cells.\textsuperscript{4,5} Protection is specific in that animals are protected only against the immunizing tumor. Immune spleen cells transferred to native animals protect them against subsequent tumor challenges.\textsuperscript{3}

While important as an initial characterization of antitumor immune reactivity, protection against subsequent challenge to tumor bears little analogy to the goal of immunotherapy of patients with progressively growing metastatic neoplasms. Subsequent work demonstrated that tumor specific immune cells could be adoptively transferred into tumor bearing hosts and could eradicate established tumors, provided that: the tumor burden was not too great, the transferred immune cells could be maintained in vivo in the tumor bearing host, and the immunizing tumor and the established tumor were the same.\textsuperscript{6,7} While these experiments provided curative immunotherapy of experimentally induced murine tumors, similar studies involving spontaneously arising murine neoplasms were notably less successful.\textsuperscript{8}

**NONIMMUNOGENIC TUMORS: A ROLE FOR NON-MAJOR HISTOCOMPATIBILITY COMPLEX-RESTRICTED CYTOTOXIC EFFECTS**

Spontaneously arising murine tumors differ from those induced experimentally; most appear to be functionally nonimmunogenic and do not appear to express tumor specific antigens that can be recognized by autologous or syngeneic cytotoxic T lymphocytes.\textsuperscript{8} Attempts at immunization with these nonimmunogenic murine tumors have been disappointing and seldom generate protective immune responses. However, these spontaneously arising, nonimmunogenic murine tumors may better reflect the clinical situation.

In most instances when patients' peripheral blood lymphocytes are examined for reactivity against autologous tumor cells, immune cells specifically able to kill autologous tumor are seldom found.\textsuperscript{9,10} While examples of apparent antigen specific reactivity to certain human tumors have been described, these may be the exception rather than the rule.\textsuperscript{11-13} Nevertheless, a distinct form of immune mediated tissue destruction not involving antigen recogni-
tion using major histocompatibility complex-restricted antigen specific receptors has been described and may be useful in destruction of nonimmunogenic neoplastic tissue.\textsuperscript{14-16}

Non-major histocompatibility complex restricted cytotoxicity was first described as a process mediated primarily by a small population of human peripheral blood lymphocytes that bore neither B nor T cell markers.\textsuperscript{17} These cells, originally designated ‘‘null cells’’ from their lack of known surface markers and absence of characterizable function, mediated destruction in vitro of certain cultured tumor cell lines. Subsequent studies of these natural killer cells produced evidence suggesting that they might play a role in responses to interferon, control of viral infection, and susceptibility to spontaneous development of certain murine tumors.\textsuperscript{18} Killing by natural killer cells, however, does not appear to involve major histocompatibility complex-restricted recognition of target cells, and no role for the $\alpha$, $\beta$/CD3 T cell receptor complex has been identified for natural killer cells.\textsuperscript{15,16} The role of these natural killer cells in the recognition and destruction of a broader range of nonimmunogenic tumor cells and the mechanism of non-major histocompatibility complex restricted recognition of target cells is still unclear.

Non-major histocompatibility complex-restricted killing is also mediated by populations of peripheral blood lymphocytes incubated in high concentrations of interleukin-2.\textsuperscript{19} Interleukin-2 is normally synthesized and released by T cells following binding of antigen to specific major histocompatibility complex-restricted T cell receptors, and it serves as a mitogenic stimulus for the subsequent proliferation of cytotoxic T cells specific for the inciting antigen.\textsuperscript{20} The interleukin-2 stimulus is mediated through an interleukin-2 receptor complex on the lymphocyte surface. This complex consists of a 55 kD chain (TAC) and a 75 kD chain (p75) which associate to form a high affinity interleukin-2 binding site. The individual chains alone also bind interleukin-2, albeit with lower affinities than the bimolecular complex.\textsuperscript{20-22} The p75 chain has been found constitutively expressed on resting T cells and natural killer cells,\textsuperscript{23-25} while TAC synthesis appears to occur concomitantly with endogenous IL-2 synthesis following antigen specific T cell receptor interactions, or following direct stimulation of the p75 chain with high concentrations of interleukin-2. Newly synthesized TAC chains then associate with p75 chains on the cell surface to form the high affinity receptor complex.

When fresh unseparated peripheral blood lymphocytes are cultured in interleukin-2, a heterogeneous population of lymphocytes is activated, presumably via the p75 chain, that mediates the non-major histocompatibility complex-restricted destruction of both natural killer cell sensitive and resis-
tant tumor cell lines, as well as fresh tumor cells. Most of the cytotoxic cells in this population bear the Leu 11 and Leu 19 surface markers. These two markers are present on natural killer cells, suggesting that the cells mediating this "lymphokine activated killer" activity (designated lymphokine-activated killer cells, with the understanding that this is a functional definition of a heterogeneous population) may have arisen from cells with natural killer activity or their precursors.

Studies in murine models demonstrated that lymphokine activated killer cells could mediate the in vivo destruction of immunogenic as well as nonimmunogenic tumors, an observation that led to speculation that lymphokine activated killer cells might be able to distinguish virtually all neoplastic from all normal tissues. However, closer inspection of the lymphokine activated killer cell phenomenon revealed that the spectrum of its activity is not limited to neoplastic tissue. Virally transformed normal tissues, hapten modified normal tissues, in vitro cultured normal tissues and even fresh normal tissues were all found to be susceptible to in vitro killing by interleukin-2 activated lymphocytes. While lymphokine activated killer cells usually mediate greater destruction of neoplastic tissue than normal tissue, the fact that normal tissue can also be destroyed indicates a potential for toxic side effects of interleukin-2 therapy. Indeed, clinical trials have confirmed this supposition (see below). Nevertheless, the quantitative differences in the degree of killing of neoplastic tissue relative to normal tissue suggest that a "therapeutic window" may exist within which immunostimulatory doses of interleukin-2 might be administered with minimal toxic side effects.

**THE LYMPHOKINE ACTIVATED KILLER CELL PHENOMENON: CHARACTERIZING INTERACTIONS BETWEEN EFFECTOR AND TARGET**

Efforts to explain the quantitative differences in susceptibility to lymphokine activated killer cell-mediated destruction observed between various target cell populations have been aimed at characterizing the mechanism of its effector/target cell interaction. While the molecular nature of these interactions is still poorly defined, studies in our laboratory have shown that populations of lymphokine activated killer effector cells that are able to mediate non-major histocompatibility complex-restricted destruction of normal autologous lymphocytes can be "competitively inhibited" or blocked by the addition of unlabeled autologous peripheral blood lymphocytes, K562 cells, Daudi cells or EBV-transformed B cells. These "cold target" blocking studies suggest that the "target molecules" on normal autologous peripheral blood lymphocytes recognized by heterogeneous populations of interleukin-2 activated cytotoxic cells are also present on the various unlabeled target cells.
Thus, the quantitative differences in destruction mediated by bulk populations of lymphokine activated killer cells do not seem to be due to specific recognition of unique determinants on the respective target cells.

Clonal analysis of individual effector cells comprising the heterogeneous population of cells mediating the lymphokine activated killer cell effect has revealed that despite the apparent lack of target specificity observed in bulk lymphokine activated killer cell cultures, a level of selectivity does exist at the clonal level that might explain the observed quantitative differences in susceptibility to killing.33 When individual lymphokine activated killer cell clones, obtained by limiting dilution plating, were studied, it was found that most clones mediated non-major histocompatibility complex-restricted killing against a variety of different targets, although the "panel of susceptible targets" for one clone was frequently different from that of another. Some clones killed K562 better than Daudi, while others killed Daudi or normal peripheral blood lymphocytes better than they killed K562. At least one clone showed greater specificity for fresh ovarian carcinoma than for the other targets. Many other patterns of target selectivity were also seen. The presence of such distinct subsets of cytolytic cells within the "nonspecific" heterogeneous bulk cultures suggests that at least some of the nonspecific cytotoxicity observed in bulk cultures of lymphokine activated killer cells may be due to the sum total of cytotoxic effects mediated by individual lymphokine activated killer cell clones with distinct target selectivities.

Cell adhesion molecules, including the lymphocyte function antigens LFA1 and LFA3 as well as laminin and the "integrin" family of adhesion molecules, appear to play a role in facilitating the approximation of target and effector cells.3,32,34,35 The apparent hierarchy of tumor target susceptibility to non-major histocompatibility complex-restricted killing could potentially be due to individual lymphokine activated killer cell clones "binding to" a different spectrum, or quantitatively different pattern, of such cell adhesion molecules. While most lymphokine activated killer effector cells display the natural killer phenotype, some clones have been identified in interleukin-2 activated bulk cultures that have uniquely rearranged T cell receptor genes and display a cytotoxic T cell phenotype.14,16,26 These T cells, despite their apparent antigen specificity, also mediate non-major histocompatibility complex-restricted killing, and appear to utilize cell adhesion molecules rather than major histocompatibility complex or antigen/T cell receptor interactions in the "recognition" of some target cells, since antibodies to the LFA1 molecule block this non-major histocompatibility complex-restricted cytotoxicity, while antibodies blocking T cell receptor or major histocompatibility complex interactions do not inhibit killing.32 The importance of
cell-adhesion molecules in the lymphokine activated killer cell phenomenon is apparent in that they enable antigen-specific T lymphocytes as well as nonspecific natural killer cells to participate in non-major histocompatibility complex-restricted killing. We have observed that most clones involved in mediating the heterogeneous lymphokine activated killer cell phenomenon show greater selectivity for transformed tumor cell lines than for fresh tumor cells or normal autologous peripheral blood lymphocytes. Whether these differences result from differences in the expression of (or interaction with) cell adhesion molecules remains unclear. Closer examination of the role of adhesion molecules in mediating target selection and a better understanding of what factors control the differential expression of individual adhesion molecules on the surface of a given target cell will be important in improving both the specificity and sensitivity of the lymphokine activated killer cell phenomenon for neoplastic tissue.

IN VIVO LYMPHOKINE ACTIVATED KILLER CELL ACTIVITY: MURINE MODELS FOR THE SYSTEMIC ADMINISTRATION OF INTERLEUKIN-2

Proceeding concurrently with the in vitro studies aimed at characterizing the lymphokine activated killer cell phenomenon were extensive preclinical in vivo studies designed to evaluate the clinical potential of interleukin-2 as a cancer treatment modality. Initial experiments in tumor-bearing mice demonstrated that the ability to produce an in vivo antitumor effect was dependent on the dose, timing, duration of interleukin-2 administered, and the degree of tumor burden. Continued maintenance of high steady-state levels of interleukin-2 appeared most effective at mediating the reduction of disseminated metastatic tumor.

Although the antitumor effects of interleukin-2 were dose-dependent, high doses of interleukin-2 also produced profound dose-dependent toxic side effects. With prolonged treatment, hypotension, wasting, diarrhea, pulmonary edema, and ascites developed, sometimes resulting in the death of the animal. These toxicities have been shown to be immune related and stem in part from a "vascular leak syndrome" following lymphokine activated killer cell mediated destruction of capillary endothelial cells.

In certain rapidly growing murine tumor models, treatment with large numbers of in vitro activated lymphokine activated killer cells (more cells than are contained in a normal mouse spleen) plus in vivo interleukin-2 is more effective than is administration of interleukin-2 alone. This suggests that the best antitumor responses to interleukin-2 will require the presence of enough activated immune cells to "get ahead" of progressively expanding tumors. Such responses might also be obtained if interleukin-2 can be
administered early in the course of disease when the tumor burden is relatively small.38 However, because the clinical application of interleukin-2 treatment is still in the early experimental stages, its use has been restricted to patients with tumors refractory to other established forms of therapy. As such, most experimental trials with this agent involve patients with large progressively growing tumors. Thus, the effectiveness of these “lymphokine activated killer cells plus interleukin-2” regimens against large tumors may depend on the number of in vitro activated lymphokine activated killer cells that can be infused. However, the in vitro growth of sufficient numbers of human lymphokine activated killer cells that should be analogous (on a per mass basis) to the number of cells found to be effective in the murine models remains difficult with present technology. Slow-growing or small tumors, on the other hand, might potentially be better treated by prolonged maintenance of constant, less toxic levels of interleukin-2 sufficient to generate and to maintain immunologic activity, rather than with a few days of high dose interleukin-2 allowing the generation of only transient in vivo lymphokine activated killer cell activity. Despite these uncertainties as to how patients would respond to the systemic administration of interleukin-2, these preclinical murine trials laid the groundwork for the initiation of clinical trials using interleukin-2 and interleukin-2 plus in vitro activated lymphokine activated killer cells in the treatment of cancer patients.

CLINICAL STUDIES

Initial Phase I trials used natural human interleukin-2 purified from mitogen-stimulated lymphocyte cultures. In two trials treating patients with the acquired immune deficiency syndrome, the low doses administered produced few toxicities but also had little effect on the immune status of these patients.42,43 Subsequent studies using somewhat higher doses of natural interleukin-2 purified from cultures of Jurkat cells (an interleukin-2 producing T cell line) resulted in minimal dose-dependent toxicities ranging from headache to nausea and vomiting, malaise, and fever and chills. Again, no antitumor responses were observed.

With the availability of recombinant human interleukin-2, clinical trials using much higher doses of interleukin-2 have been possible. Significant dose-related toxicities have been observed (see below) which have been the major limiting factor in treatment.

In initial clinical investigations using recombinant interleukin-2 at doses greater than 10^6 units/m^2/day (given three times a day intravenously) fever, chills, capillary leakage, pulmonary edema, dermatitis, hepatocellular in-
jury, and renal dysfunction were all observed\textsuperscript{46,47} and were similar to the toxicities previously observed in the murine models. These toxicities quickly disappeared upon cessation of interleukin-2 therapy. Lymphocyte counts from these patients increased following the course of therapy as did the in vitro proliferative responsiveness of these peripheral blood lymphocytes to interleukin-2. However, the peripheral blood lymphocytes showed little in vitro lymphokine activated killer cell activity (against natural killer cell-resistant targets) and no antitumor responses were observed.

Subsequent clinical testing involved increased doses of interleukin-2 together with lymphokine activated killer cells grown exogenously in interleukin-2. Studies at the National Cancer Institute documented that doses of interleukin-2 greater than $10^7$ units/m\textsuperscript{2}/day could be administered, although with significant toxicity, often necessitating intensive care unit monitoring with respiratory and pressor support. Results of this regimen of infusion of lymphokine activated killer cells and five days of high-dose interleukin-2 given as intravenous bolus injections three times a day were presented in an initial report documenting in vivo antitumor effects in 11 of 25 patients treated.\textsuperscript{48} Subsequent collaborative studies utilizing this treatment protocol have shown that approximately 20\% of patients with renal cell carcinoma or malignant melanoma have measurable (>50\%) shrinkage of all tumor.\textsuperscript{13,49,50} For some of these patients complete regression of tumor was observed, and while many have experienced progressive regrowth of tumor, at least some have sustained remissions for one year or more.

These exciting studies document that an entirely immunologic approach can have a dramatic antitumor effect for at least some patients. Nevertheless, only a small minority of patients show any clinical benefit, while most patients treated with these high doses of interleukin-2 have considerable and often life-threatening toxicities. Further improvements, namely, better antitumor effects with less toxicity, are essential. We believe that the continued improvement of interleukin-2 as a therapeutic modality will depend on careful monitoring of the immunologic changes effected by interleukin-2. Continued reexamination of dose and administration regimens, coupled with the combined use of interleukin-2 with other treatment modalities may be essential to enable more effective and widespread application of this approach to immunotherapy.

**Phase I Trials at the University of Wisconsin: Responses to Immunotherapy with Acceptable Toxicity**

In our initial study we sought to establish a way to administer inter-
leukin-2 such that immunologically significant levels could be maintained in vivo.51,52 The 25 patients enrolled in that Phase I trial received escalating doses of interleukin-2, either as daily bolus injections or continuous infusions, for seven days. Patients tolerated doses of interleukin-2 up to $3.0 \times 10^6$ units/m$^2$/day, while $10^7$ units was not well tolerated. Toxicities observed were similar to but less severe than those described when interleukin-2 was provided three times a day at greater than $10^7$ units/m$^2$/day. No patients in our study required intensive care unit monitoring. During infusion, patients developed a profound dose-dependent lymphopenia.52 Peripheral blood lymphocytes obtained during this period of lymphopenia had decreased in vitro proliferative responses to interleukin-2 as well as decreased in vitro natural killer and lymphokine activated killer cell activity.53 The observation that these residual circulating lymphocytes, when mixed with pretreatment peripheral blood lymphocytes, did not suppress the natural killer, lymphokine activated killer cell, or interleukin-2 proliferative responses of the pretreatment lymphocytes suggested that during interleukin-2 infusion, interleukin-2 responsive cells were selectively disappearing from the peripheral circulation.54 Upon cessation of interleukin-2 therapy, a transient rebound lymphocytosis was observed during which lymphocyte counts exceeded pretreatment baseline levels. Peripheral blood lymphocytes obtained during this rebound period showed increased natural killer and lymphokine activated killer cell activity, augmented interleukin-2 responsiveness, and an increase in lymphocyte activation markers. All of these changes were dose dependent such that the greater than interleukin-2 dose given to patients, the greater the in vitro responsiveness to interleukin-2. Furthermore, a comparison of bolus injections versus continuous infusion revealed greater immunologic activation and a greater lymphocyte rebound in patients receiving continuous infusion.52 Despite the striking immunologic changes observed, none of the 25 patients treated experienced a measurable antitumor response.

In this initial study the state of immunologic activation generated by interleukin-2 rapidly waned within the two-three days of stopping treatment. This observation served as a basis for a subsequent Phase I trial testing our hypothesis that prolongation of this in vivo activated state could potentially generate a greater antitumor effect. In this recently completed study, 28 patients were scheduled to receive interleukin-2 either at 1.0 or $3.0 \times 10^6$ units/m$^2$/day. Interleukin-2 was administered over the course of four weeks for four consecutive days each week followed by three days rest. Interleukin-2 was given by daily bolus injection, continuous infusion, or a combination of continuous infusion together with daily bolus injection.
Immunologic changes observed were similar to those seen in our initial study. However, the state of immune activation continued to increase throughout the four-week course of therapy. Peak rebound lymphocyte counts following each week of interleukin-2 showed progressive increases. At the end of four weeks of therapy, patients receiving $3.0 \times 10^6$ units/m$^2$/day had nearly a 10-fold increase in circulating lymphocytes relative to pretreatment baseline levels. Lymphocytes obtained after four weeks also showed a dramatic increase in interleukin-2 dependent lymphokine activated killer cell activity in vitro, with patients receiving $3.0 \times 10^6$ units/m$^2$/day showing more than a 150-fold increase in "circulating cytotoxic potential." Treatment was reasonably well tolerated and no patients enrolled in this study experienced life-threatening toxicity despite significant malaise, fever, nausea, and dermatitis in most. No patient showed signs of significant hepatic or renal toxicity (although one patient had a transient creatinine elevation of >3.0 mg/dl) and none required pressors, respiratory support, or intensive care unit monitoring.

Antitumor effects using this regimen were observed. None of the patients receiving the lower dose ($1.0 \times 10^6$ units/m$^2$/day) or interleukin-2 by bolus injection (at either dose) responded. In contrast, of the 18 patients receiving $3.0 \times 10^6$ units/m$^2$/day, three showed measurable, although transient, responses with greater than 50% shrinkage of all measurable tumor. One patient of 14 receiving $3 \times 10^6$ units/m$^2$/day by continuous infusion showed an antitumor response, and two of four patients receiving the combined regimen of continuous infusion plus daily bolus injections had a response. Each of the three responding patients had renal cell carcinoma. Although the differences in response rate between these groups (one of 14 versus two of four) were not found to be statistically significant ($p=0.11$), possibly because of the small number of patients studied, the data are provocative and suggest that further clinical testing of the combined schedule is warranted.

**IN VITRO MONITORING OF IN VIVO LYMPHOID ACTIVATION**

We have sought ways of quantitating the level of in vivo immune activity during interleukin-2 infusions despite the lymphopenia and lack of circulating interleukin-2 reactive cells at these times. Lotze et al. initially observed that in vivo treatment with interleukin-2 resulted in the release of soluble forms of the TAC molecule of the interleukin-2 receptor complex into the plasma. Other studies have also correlated increases in serum interleukin-2 receptor level with endogenous cellular immune activity. We have shown...
that the levels of receptor released into the plasma during infusions are far
greater than can be accounted for by the activation of the lymphocytes circu-
lating at that time.\textsuperscript{60} We have suggested that serum interleukin-2 receptor
levels reflect interleukin-2 activation of both circulating and noncirculating
lymphoid elements, and are an effective way to assess total lymphoid mass
activation. Experiments are underway to determine whether there is a statisti-
cally significant relationship between the levels of interleukin-2 receptor
released and the dose or route of administration. Preliminary data in this
regard show relatively lower and more erratic patterns of interleukin-2 recep-
tor release in patients receiving daily bolus injections, as compared to contin-
uous infusion, and are consistent with the less striking changes in other
immunologic parameters observed in those patients (Voss and Sondel, unpub-
lished).

Our recent Phase I study indicated that four weeks of continued inter-
leukin-2 therapy had greater immunologic and antitumor effects than did one
week of therapy.\textsuperscript{55-57} It is not clear, however, whether continued therapy at
that time would have provided additional benefit and further augmented
immunologic activity, or if the in vivo responses to interleukin-2 reach a
plateau after four weeks. In in vitro studies we have observed that culturing
fresh peripheral blood lymphocytes in successively higher concentrations of
interleukin-2 (up to 1,000 units/ml) produced dose-dependent increases in
lymphokine activated killer cell activity (Figure 2). Nevertheless, after in
vitro stimulation, the levels of interleukin-2 receptor (TAC) synthesis (mea-
sured in whole peripheral blood lymphocyte lysates) and levels of TAC
released into culture supernatants showed an interleukin-2 dose-dependent
increase only at interleukin-2 concentrations less than 100 units/ml; unlike the
cytotoxic response, this response plateaued at concentrations $\geq$ 100 units/ml
(Figure 1).

While the steady-state serum levels of interleukin-2 attainable (without
life-threatening toxicity) in patients (30 units/ml IL-2) are lower than these in
vitro concentrations used in the above-described studies, it is conceivable
that continuous infusion to generate steady state levels sufficient to maintain
activated proliferating interleukin-2-responsive immune cells coupled with
bolus injections to generate "bursts" of in vivo cytolytic activity might be
more effective than simply maintaining populations of immune cells with
significant "untapped reserves" of cytolytic activity. Our clinical and labora-

tory data support this concept; further testing of the "combined" regimen
is underway. One possible approach might be to establish a maintenance dose
of interleukin-2 sufficient to generate significant systemic immunologic ac-

Vol. 65, No. 1, January, 1989
Fig. 1. Soluble interleukin-2 receptor (IL-2R = TAC) release as a function of concentration. Fresh peripheral blood lymphocytes were cultured in interleukin-2 at 30, 100, 500, or 1,000 units ml or in culture medium alone. Cell-free supernatants from parallel cultures were assayed on days three, four, and five for levels of soluble interleukin-2 receptor. Levels are expressed as units released per 10⁶ cells cultured.

tivity, yet at which toxicity is tolerable, and then provide additional local bolus injections directly into the tumor environment or, alternatively, via intralymphatic infusions aimed at metastatic tumor. There is some precedent for anticipating a response from both local interleukin-2 injections and intralymphatic infusion.61,62 The establishment of populations of activated cytotoxic lymphocytes, poised and awaiting a subsequent bolus of interleukin-2, might have synergistic effects greater than either regimen alone. Furthermore, provision of interleukin-2 locally might not dramatically add to the toxicities already generated by the constant infusion.

We have also observed that populations of peripheral blood lymphocytes depleted of TAC-bearing cells (TAC+) proliferate in response to interleukin-2 and mediate lymphokine activated killing just as well as parallel cultures of unseparated peripheral blood lymphocytes. This is consistent with work from other groups showing that interleukin-2 binding just to the p75 chain of the interleukin-2 receptor complex (constitutively expressed on some natural killer cells and resting T cells) could generate cytolytic activity, while proliferation of the cells depended on the synthesis and expression of
Fig. 2. Lymphokine activated killing (cytotoxicity against Daudi target) as a function of interleukin-2 concentration. Fresh peripheral blood lymphocytes were cultured in the respective concentrations of interleukin-2 or medium alone for five days. On day five, cells were collected and tested against Daudi targets in the chromium-release assay. Effector cells were resuspended in the same medium from which they had been collected. No fresh interleukin-2 was added to the cytotoxicity assay. Cytotoxicity is expressed as lytic units, with one lytic unit defined as the number of effector cells causing 20% lysis of $5 \times 10^3$ target cells; lytic units (LU) are expressed as LU/10^7 effector cells. These data are representative of multiple experiments carried out with peripheral blood lymphocytes from various healthy donors (for methodological details).^30,60^

the TAC molecule on the surface,^23-25^ presumably in the form of a high affinity complex with the p75 chain. In preliminary studies, we have observed that peripheral blood lymphocytes depleted of TAC positive cells and cultured in interleukin-2 remain TAC negative (based on flow cytometric analysis) and consist predominantly of cells bearing the Leu 19 natural killer cell-associated molecule. In addition, whereas significant levels of soluble TAC are released into the supernatants of unseparated peripheral blood lymphocyte cultures during growth in interleukin-2, levels of the TAC molecule released from the TAC depleted populations are very low, despite the proliferation and killing exhibited by these TAC negative cells (Weil-Hillman et al., unpublished).

That T cells seem to require at least the transient presence of TAC on the cell surface to proliferate, combined with our preliminary observation suggesting that Leu19+ TAC negative cells continue to proliferate without re-
leasing significant quantities of soluble TAC, suggests that some natural killer cells may process the interleukin-2 signal differently than other lymphocyte populations. In light of these preliminary data, more work is needed to establish the origin of soluble interleukin-2 receptors released into the plasma of patients receiving interleukin-2. Because heterogeneous natural killer and T-cell elements are being activated in vivo by interleukin-2, it remains possible that natural killer-like cells contribute the bulk of lymphokine activated killer cell activity, while the co-activation of TAC positive, T3+ T cells and their in vivo release of soluble TAC has provided us with a means of directly monitoring the state of total lymphoid activation.

PROSPECTS FOR FUTURE APPLICATIONS OF INTERLEUKIN-2 THERAPY

Our clinical investigations together with data from other clinical teams document that interleukin-2 can induce significant in vivo lymphokine activated killer cell activity without necessarily producing life-threatening toxicity. However, while antitumor effects have been observed, the rate of response has been disappointingly low. As future clinical investigations progress, we have emphasized the importance of continued basic research into the molecular mechanisms by which interleukin-2 activates both proliferative and cytotoxic responses, as well as the means through which effector cells, once activated, recognize target cells and exert their cytotoxic effects. In addition to these basic studies, preclinical data suggest that interleukin-2 therapy combined with other biological response modifiers (including interleukin-1, interleukin-4, tumor necrosis factor and interferons alpha, beta and gamma), monoclonal antibodies directed against tumor-associated antigens, or pretreatment chemotherapy to reduce tumor burden may result in synergistic antitumor responses. Clinical trials investigating some of these approaches are underway. Because of the rapidity with which interesting and exciting new approaches to cancer immunotherapy are being developed, the number of potential combination therapies is increasing enormously. Nevertheless, the successful development of effective forms of combination therapy will first require establishing safer and better tolerated interleukin-2 treatment regimens to which these other treatments can be added. Further improvements are essential if immunotherapy regimens using interleukin-2 are to provide a therapeutic benefit for a significant fraction of cancer patients and become included as a part of standard oncologic care.

ACKNOWLEDGEMENTS

The authors thank Drs. Peter C. Kohler, Barry Storer and Ernest C. Bor-
den, as well as Nan Miller, Karen-Husby Moore, Aggie Borchert, Robin Bechhofer, Catherine Nobis, Diana Wondrash, and Kathy Schell for their major roles in completing clinical, laboratory, and statistical aspects of this study, and K. Edge for preparation of this manuscript. All patients entered into clinical trials at the University of Wisconsin and all healthy blood donors signed consent forms approved by its Committee for the Protection of Human Subjects.

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