Interleukin-2 and Lymphokine Activated Killer Cells: Promises and Cautions

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ABSTRACT

The purposes of this work are to: review the biological activities of Interleukin-2 (IL-2); evaluate the reported therapeutic benefits and toxicity of IL-2/lymphokine activated killer (LAK) cells; and project the role of IL-2/LAK cells in cancer therapy. Interleukin-2 is a glycoprotein lymphokine (mw 15,000) produced naturally by mitogen or antigen stimulated T-lymphocytes. The activities of IL-2 include: enhancement of IL-2 receptor positive T-lymphocytes and a variety of other in vitro and in vivo alterations of T cell function. The IL-2 gene has been cloned from the Jurkat leukemia cell line and expressed by recombinant biotechnology in an E. coli vector. In vitro incubation of IL-2 with selected T-lymphocytes results in the formation of lymphocyte activated killer (LAK) cells. Rosenberg and colleagues, in 1983, demonstrated that both exogenous IL-2 and LAK cells were needed in order to get maximum tumor regression in a murine model and later humans. Patients selected for IL-2/LAK cell therapy have clinical metastases or advanced unresectable cancers. Almost all patients treated demonstrate some toxic effects, including chills, fever, nausea, vomiting, diarrhea and hepatic dysfunction. Approximately 75 percent of the patients have profound hypotension and require intensive nursing care. A review of the literature indicates that tumor responsiveness will range from negligible (adenocarcinoma of the lung with metastases) to a 30 + percent response in renal cell carcinoma when complete and partial responders are totalled. Interleukin-2/LAK cell therapy has promise for some wide spread tumors for which no other therapy is available. There are significant side effects and toxicity, as well as technical problems, associated with IL-2/LAK therapy.

Introduction

Lymphokine-activated killer cells (LAK) were first described in the early 1980’s. Grimm and colleagues, in 1982, described a method of incubating human peripheral lymphocytes with Interleukin-2 (IL-2) and the resulting effect that these lymphocytes had on tissues.4 They recognized that these activated killer lymphocytes would lyse fresh tumor cells in short-term chromium release studies. Rosenberg et al described in vivo antitumor activity of this adoptive
immunotherapy in a variety of patients with metastatic carcinomas. Unlike other killer cells (natural killer (NK) or T-cytotoxic cells), Rosenberg et al reported that LAK cells could be produced in normal, as well as tumor-bearing, patients and that the killer activity was not restricted to histocompatibility loci in regard to target cell specificity.

Interleukin-2 is a glycoprotein of approximately 15,000 daltons which can be produced after mitogen or specific antigenic stimulation of certain presensitized T-lymphocytes. Interleukin-2 is a lymphokine which expands T-cell subgroups, particularly those with IL-2 receptors. In vitro, IL-2 maintains T-cells in long-term cultures, enhances helper and cytotoxic T-cell reactions to a variety of antigens, and augments the generation of cell mediated cytotoxic T-cell activity. The in vivo activity of IL-2 includes the enhancement of NK cell functions, the augmentation of T-cell alloantigenic responses, and the improvement of the immune function in patients with congenital and acquired immunologic states under experimental conditions.

Interleukin-2 can be produced by recombinant gene technology using an IL-2 gene from the Jurkat leukemia line cloned and expressed at high levels in an Escherichia coli vector. This type of IL-2 is known as recombinant IL-2 (rIL-2). Recombinant IL-2 has a serum half-life of approximately 2 to 3 minutes. It is cleared and/or metabolized in the renal tubules if given intravenously. These data have been obtained from observations in mice. In the same experimental animal, an intraperitoneal injection of recombinant IL-2 has a half-life of approximately two to three hours. Recombinant IL-2 given to humans as an intravenous bolus has a biological half-life of approximately six to 10 minutes. The LAK cells are produced by incubation of normal or stimulated T-lymphocytes with either recombinant or natural IL-2 LAK precursors which are generated or enhanced by systemic administration of IL-2 and are highly active after in vitro IL-2 incubation.

Maximum LAK cell activity results after three or four days of incubation with appropriate amounts of IL-2. Purified recombinant IL-2 alone is sufficient to transform lymphocytes to tumoricidal activity. Once activated, LAK cells are lytic for a broad range of autologous and allogeneic tumor cells, but they do not lyse normal allogeneic cells, such as lymphocytes, lung, or liver cells. The T-lymphocytes that are able to be transformed into LAK cells express Leu 1+, OKT 3+, 4−, 8+ antigenic markers on their cell surface. The exact mechanism whereby lysis of cells occurs through the action of LAK cells is not known, but it is proposed that it is different from the action of NK cells or those of the classic cytotoxic T-lymphocytes.

Treatment

Muul and colleagues, in 1984, demonstrated that recombinant IL-2 given intraperitoneally in a murine model resulted in decreased metastatic tumor nodules in the lung. Both exogenous IL-2 and LAK were needed to get maximum tumor regression. Early human studies showed no significant clinical responses in 65 patients given LAK cells alone or IL-2 alone in doses up to 30,000 units per kilogram on a three times per day basis. These findings led to the use of a combination of IL-2 and LAK cells for the treatment protocols.

A protocol which has been used in human treatment is given. One hundred thousand units of rIL-2 per kilogram are given intravenously every eight hours for four to five days. The patient is then subjected to lymphocytapheresis 48 hours after the last dose on a daily basis for the four or five days of rIL-2 treat-
ment. Lymphocytes obtained by the apheresis procedure are then incubated in vitro for three to four days at 37°C with additional rIL-2. The activated cells are harvested from culture and then washed and returned to the patient by intravenous bolus. The intravenous rIL-2 is re-started at the time of the first LAK infusion and continued for three to four days to potentiate additional LAK cell activity in vivo. The average hospital stay for patients treated with this regimen is approximately 21 days.

Patients selected for IL-2/LAK cell therapy, also known as adoptive immunotherapy, are selected on the basis of the presence of metastases or advanced unresectable carcinomas. The patients generally have not responded to standard therapy or the tumors are not amenable to surgical resection. Most of the patients have had adenocarcinoma of the kidney, adenocarcinoma of the colon and rectum, but there have also been patients treated with central nervous system tumors, sarcomas and even melanomas.

Results of Therapy

The results of therapy in limited numbers of patients indicate that complete or partial responses are seen in a variety of cancers or lymphomas. A complete response refers to disappearance of all measurable tumor. A partial response refers to greater than or equal to a 50 percent decrease in the size of the sum of all measurable lesions. Using these criteria, approximately 30 percent of the patients with adenocarcinoma of the kidney, 23 percent of the patients with melanoma, and 12 percent of patients with colorectal adenocarcinoma were said to have either a complete or partial response. In a very small number of patients reported in Rosenberg’s study, two out of two patients with non-Hodgkins lymphomas had responses, one of which was a complete response and the other a partial response. In those patients that have had complete responses, the responses have been durable and long-lived, that is continuing beyond one year. Fisher et al in a multi-center study arrived at the conclusion that IL-2/LAK cell therapy may be the treatment of choice at present for metastatic renal cell adenocarcinoma.

Early results of the use of LAK cells injected directly into glial tumors has yielded encouraging results with 33 percent of a small number of patients responding to therapy. Direct injection of LAK cells into sarcomas, or other late metastasizing tumors, has not been evaluated, but may offer a future application.

Toxicity and Complications

Almost all patients experience some chills and fever. Nausea, vomiting and diarrhea are almost universal complaints from the patients who are treated. Hepatic dysfunction, particularly elevations of the bilirubin and the transaminases, are noted in almost all patients. Many patients have a diffuse erythematous, and sometimes pruritic, rash. Skin desquamation is uncommon. Profound hypotension has been reported in as many as 75 percent of the patients. This occasionally requires special nursing care, including central venous pressure monitoring. It is always advisable that patients being treated with this mode of therapy have special nursing care and monitoring available to them. It is thought that this profound hypotension is secondary to capillary leaking or increased permeability syndrome. This results in a decreased intravascular volume and the associated hypotension. Because of the increased permeability of the capillary, approximately 33 percent of the patients also have significant fluid retention with an increase of 10 percent or more of their body weight. Pulmonary
interstitial edema has been reported in a small percentage of the patients, along with respiratory insufficiency and problems requiring mechanical ventilation. Cardiac (usually atrial) arrhythmias and rare myocardial infarcts have also been reported. The cardiovascular and renal vascular complications, including azotemia and oliguria, are thought to be associated with the severe hypotension. A variety of problems associated with altered mentation, somnolence, disorientation, overt psychosis, coma and headaches have been reported (table I).

Hematologic effects associated with IL-2/LAK cell therapy are common. The majority of patients develop pancytopenia with approximately 88 percent of the patients requiring red cell transfusions for anemia. Almost 75 percent of the patients have thrombocytopenia with platelet counts of less than 100,000 per microliter. Some patients have platelet counts that drop to less than 20,000 platelets per microliter. Platelet counts usually return to close to pre-therapy levels within two weeks after the discontinuation of IL-2. Abnormal coagulation studies have been reported in approximately 33 percent of the patients. These are related to decreased Factor VII levels and have responded to Vitamin K administration. It is thought that these abnormalities are related to the interference of metabolic pathways of the liver involved in the production of these protein coagulation factors. The coagulation abnormalities have also been attributed to poor nutrition, intravenous antibiotics, or hemodilution. Lymphocytopenia occurs in almost all patients during therapy, but returns to normal, or even elevated levels, within 48 hours of discontinuing IL-2 (rebound lymphocytosis). The postulated mechanism for the rapid fall and subsequent rapid recovery of lymphocyte counts is that this may be secondary to sequestration and increased adherence of lymphocytes to vascular endothelium, although there is no good experimental documentation of this. No significant changes in the neutrophil count are usually noted. Some of the effects noted in the peripheral blood counts may be associated with the in vitro activity of IL-2 on colony-forming cells of the hemopoietic system. High concentrations of rIL-2 result in inhibition of erythroid colony growth.

**Conclusion**

Recombinant IL-2 is available in reasonably large quantities now as a product of biotechnology. Interleukin-2 is used in direct therapy and in the production of lymphokine activated killer cells in patients with a variety of tumors. The IL-2/LAK cell therapy has promise in tumors for which no other therapy is presently available. The tumors which

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**TABLE I**

Adverse Effects of Interleukin-2 and Lymphokine Activated Killer Cells

<table>
<thead>
<tr>
<th>&gt; 75% of Patients</th>
<th>Chills</th>
<th>Fever</th>
<th>Nausea</th>
<th>Vomiting</th>
<th>Diarrhea</th>
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<tbody>
<tr>
<td></td>
<td>Hepatic Dysfunction</td>
<td>Increased Bilirubin</td>
<td>Elevated Transaminases</td>
<td>Pancytopenia</td>
<td>Anemia</td>
</tr>
<tr>
<td>25% to 75% of Patients</td>
<td>Profound hypotension</td>
<td>Skin manifestations</td>
<td>Erythema</td>
<td>Puritis</td>
<td>Maculopapular eruptions</td>
</tr>
<tr>
<td>&lt; 25% of Patients</td>
<td>Pulmonary interstitial edema</td>
<td>Respiratory insufficiency requiring mechanical ventilation</td>
<td>Cardiac arrhythmias</td>
<td>Renal vascular problems</td>
<td>Azotemia</td>
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have been treated tend to be those of the adenocarcinoma and the non-Hodgkin's lymphoma type. Ongoing studies are attempting to identify other responsive malignancies. There are preliminary reports of responses for central nervous system tumors. It is apparent that IL-2/LAK cell therapy is associated with significant side effects and toxicity. The side effects and toxicity are such that the use of IL-2/LAK cell therapy should be limited at the present time. The technical difficulties in caring for the patients, preparing the LAK cells, and having the available nursing, clinical laboratory science and clinical staff for support of these patients is an expensive and a significant impact on hospital resources. The IL-2/LAK cell therapy shows great promise for some tumors. Between 10 and 30 percent of specifically selected tumors showing some response. It is the current treatment of choice for metastatic renal cell cancer. However, the wide variety, serious significance of toxicity, and cost in terms of personnel and hospital resources, must give rise to caution for its use at the present time.

References