Paul M. Sondel, M.D., Ph.D., Hiroshi Kawai, M.D., Ph.D. and Osvaldo H. Westly, M.D., Departments of Pediatrics, Human Oncology, and Genetics, University of Wisconsin, 53792

Introduction

Interleukin-2 is a potent hormone of the immune system that has clear immunostimulatory activities through its effect on lymphocytes and other tissues. Of particular importance, is the response to interleukin-2 by T lymphocytes and natural killer cells. A variety of clinical protocols utilizing interleukin-2 has shown clear, reproducible, antitumor effects in some patients with certain malignancies, particularly renal cell carcinoma and melanoma.

Multiple animal models have documented that interleukin-2 can have potent therapeutically beneficial effects in several murine leukemia systems (reviewed in 1,2). Some systems utilize IL-2 as single agent therapy to treat animals with minimal amounts of leukemia while others combine IL-2 with cellular infusions, other chemotherapy, or other biologic mediators (cytokines). In each of these model systems, careful attention to specific dose, timing, and other variables have enabled generation of specific protocols that can be highly effective in tumor eradication in each model. However, the specific protocols used for each of these animal models are often quite different. An IL-2 dose, schedule, or regimen that can be effective for treating one murine tumor in one strain will not have the same result in another strain. In part, this reflects the ability of IL-2 to act in these systems on a variety of lymphocyte populations (α/β receptor bearing T cells that are autologous or allogeneic, γδ T cells, or natural killer cells).

In extrapolating from these well characterized animal models to the clinical setting, each individual patient’s tumor, immune system, and susceptibility to immune toxicity are distinct from one another. Thus, each patient appears to be his or her own "experimental model."

Designing effective immunologic therapies that are clinically relevant could therefore require developing those in vitro analytic assays to characterize which component of the immune response is most important in any single patient’s immunotherapeutic capabilities, and then using these data to design a protocol specifically focused on that patient’s immune-tumor interaction. Alternatively, an immunotherapeutic protocol that could be broadly applicable to all patients, regardless of the unique features of their individual unique tumor and immune system capacities, would have far greater applicability.

The purpose of this brief report is to summarize clinical and in vitro work from our laboratory relevant to these issues.

Lymphocyte Recognition of Hematologic Tumor In Vitro

Recognition by Autologous α/β T Cell Receptor Bearing T Cells

In multiple murine systems, the most potent population of immune cells able to mediate antigen specific responses and provide in vivo protection are the helper and killer cell populations of T cells bearing α/β T cell receptors. The exquisite specificity of this α/β T cell receptor to recognize viral or tumor specific peptide in a MHC restricted way provides a level of specificity that is efficacious and potent. Analyses of T cell responses to autologous melanoma have, for some patients, demonstrated autologous T cell reactivity. Similar studies have proven positive for T cells for patients with HTLV associated leukemia, particularly in Japan. However, for most other malignancies, including hematologic malignancies, a variety of in vitro culturing techniques have not reproducibly generated T cell reactivity that was clearly tumor specific (3).

In prior analyses of lymphocyte reactivity to autologous leukemia cells, the inclusion of “third party stimulus” suggested that providing necessary helper activity might boost T cell reactivity to autologous leukemia (4). However, in retrospect, much of the antileukemic activity induced in such systems may have been the result of IL-2 production yielding activation of NK cells to mediate the lymphokine activated killer (LAK) cell phenomenon (reviewed in 5).

Detailed studies in our laboratory of a patient with acute T helper cell leukemia showed that culturing the leukemic population with IL-2 in vitro enabled the outgrowth of a contaminating normal T cell population comprising less than 5% of the initial leukemic preparation, but “taking over” the culture after a few weeks. This cytotoxic T cell line could mediate specific destruction of the autologous leukemia cells and recognize class I determinants, using an α/β T cell receptor recognition mechanism (6). Similar analyses of ten other patients (Sosman, J., Oettel, K., and Sondel, P.M., unpublished data) with T cell leukemia showed no comparable reactivity with nine of them, and weak cytoxicity in the tenth that may have been somewhat analogous to that in this initial patient. This difficulty in detecting immune reactivity to autologous leukemia cells suggests that:

1. the leukemia cells lack a leukemia "associated" immunogenic peptide (a prospect that seems unlikely given the multiple genetic changes associated with hematologic malignancies); or
2. something about the leukemia associated peptide or the MHC antigen of the leukemic cell makes it a weak immunogen; or
3. the immune response repertoire of the patient does not provide the ability to respond to the immunogen.

Reactivity by Allogeneic T Cells

If leukemia cells bear an immunogenic peptide appropriately presented by MHC, but are poorly recognized because of an immune response defect (inherited or acquired) by the patient, then the use of allogeneic T cells may enable detection of leukemic specific reactivity. This may account for the GVL (graft vs leukemia) effect so well characterized in mouse, and documented repeatedly in clinical studies (7). A clonal analysis of this possibility has been performed utilizing HLA disparate lymphocytes in response to irradiated leukemia cells (8). Limiting dilution clonal analysis provided multiple clones that showed quantitative yet not qualitative leukemia specificity (9). It remains uncertain whether this specificity resulted from recognition of a leukemia specific peptide found only on the leukemia cells, or recognition of an alloantigen shared by leukemia cells and remission cells, with destruction of the leukemia cells preferentially because of other leukemia related adhesion or susceptibility molecules.

In further elucidating the possibility of T cell recognition of specific leukemic peptides, we are presently evaluating the ability of generating clonal populations of T cells able to recognize the fusion peptide resultant from the 9→22 Philadelphia Chromosome translocation found in Chronic Myelogenous Leukemia. In recently published murine studies(14) this fusion peptide has been found to be immunogenic.

While this approach may prove very useful in clarifying what molecules might be identified on leukemia cell surfaces that could provide immune specific targets, the prospects for designing a clinical protocol in which such clones have been screened for, and expanded, so they could be infused in large number, seems somewhat far off although not totally impossible or impractical.

Recognition by γδ T Lymphocytes

To better characterize the non-MHC component of the immune reactivity to hematopoietic cells, Paul Fisch has cloned lymphocytes in the presence IL-2 to generate NK clones and evaluate specific recognition mechanisms they use to potentially account for the different specificity patterns seen
with different clones different tumor cells (10). This approach generated several NK clones but many γδ T cell clones that mediated non-MHC restricted killing. Interestingly, this killing showed a different specificity pattern, with strong destruction of Daudi and K562 targets (like NK clones), but weak or no killing of the Raji cells, which were readily destroyed by NK clones from the same donors (11). Detailed subsequent studies have documented that these γδ cells apparently use their T cell receptor to recognize a 61 KD heat shock protein that is analogous to the GroEL family of heat shock proteins, and expressed on the surface of the Daudi cells. This determinant is cross reactive with an antigen found in mycobacterial extracts and is preferentially recognized by the γδ subpopulation utilizing the Vγ9/Vδ2 receptor subtypes (12).

**Antibody Facilitated Lymphocyte Mediated Tumor Destruction**

Multiple clinical studies have documented that IL-2 infusions can dramatically augment NK numbers and function. However, only a minority of patients are showing measurable tumor shrinkage in response to IL-2. Greater doses of IL-2 clearly cause greater toxicity without necessarily causing a dramatic difference in antitumor response. We hypothesize that greater antitumor effects requires greater specificity of the lymphocyte tumor interaction. In vitro this can be accomplished utilizing antibody that can mediate antibody dependent cellular cytotoxicity (ADCC). Specifically, lymphocytes obtained before and after in vivo IL-2 therapy show a dramatic augmentation in their ability to destroy tumor cells in the presence of tumor specific antibodies able to facilitate ADCC (13).

**Clinical Application of In Vitro Principles**

Preliminary data from a number of centers suggest some direct in vivo antitumor effect can be obtained from IL-2 treatment. Studies underway at present, will demonstrate with randomized controlled determinations, whether IL-2 as single agent therapy can have an antitumor effect in the minimal residual disease setting, where animal models predict it will be most effective. These results should be forthcoming soon.

Recent in vivo and in vitro studies (17), suggest that IL-2 administration following allogeneic bone marrow transplantation may enhance the antileukemic potential of the allograft. In addition, other observers (18) have alluded to the possibility of other cytokines as well as CMV seropositivity as modulating factors involved in the generation of an antileukemic effect by the donated marrow functioning in the recipient.

However, several preclinical analyses suggest that the antitumor effects of IL-2 may be more complex than may be obtained by infusion of IL-2 as single agent therapy. Whether the desired effects are predominantly through T cells versus NK cells, whether they require alloantigen recognition or not, whether allogeneic or autologous NK cells can appropriately destroy a patient's leukemia (15,16,19), and whether the leukemia cell expresses the appropriate adhesion molecules to enable its recognition by activated lymphocytes are all issues of great concern, and factors for which individual patients may vary. Thus, even if IL-2 can have an antitumor effect in some patients, it is possible that the number of patients benefiting from IL-2 might not be substantially large enough to give a statistically positive result in a randomized controlled clinical trial.

Furthermore, in vitro and in vivo murine studies document that combined approaches utilizing IL-2 can have very potent antitumor effects that should be testable in the clinical setting. It is for this reason that individual laboratories and clinical research teams should continue pursuing unique, state-of-the-art, in vitro dissections to help characterize and harness the antitumor effects of these different lymphocyte populations, and perform innovative "pilot" clinical protocols attempting to combine in vivo effecter cell activation with IL-2, together with other approaches that could be synergistic. In this way, regardless of the results of the ongoing randomized clinical trials of IL-2 as single agent therapy, subsequent studies should be able to build on those results to generate, hopefully, more effective treatment regimens.

**Acknowledgements**

The authors thank Jeff Sosman, Jacquelyn Hank, Mark Albertini, and Vera Malkovska for helpful discussions, Mary Pankratz and Bonnie Rayho for preparation of this manuscript. Thanks are also given to the many research teams providing critical analyses of the Graft versus Leukemia phenomenon, and of IL-2 as potential therapy for hematologic malignancy; space limitations prevent a detailed review or referencing of this extensive literature.

**References**


