0031-6997/90/4201-0001$02.00/0
PHARMACOLOGICAL REVIEWS

Vol. 42, No. 1
Printed in U.S.A.

Human Recombinant Interleukin-2 as an Experimental Therapeutic

JEFFREY L. WINKELHAKE* AND STACEY S. GAUNY

Department of Pharmacology, Cetus Corporation, Emeryville, California

*To whom requests for reprints should be addressed: Department of Pharmacology, Cetus Corporation, Emeryville, CA 94608.

†Abbreviations: (r)IL-2, (recombinant) interleukin-2; i.p., intraperitoneal; i.v., intravenous; s.c., subcutaneous; NK, natural killer; CTL, cytotoxic T lymphocyte; LAK, lymphokine-activated killer; IFN, interferon; Ts, suppressor T cell; HEV, high endothelial venule; VLS, vascular leak syndrome; MTD, maximum tolerate dose; BCG, bacillus Calmette-Guerin vaccine; (r)TNF, (recombinant) tumor necrosis factor; CTX, cytoxan.

I. Pharmacological Challenges of a Novel Protein Drug

Since its identification by Morgan et al. (1976), T cell growth factor or interleukin-2 (IL-2)† has been studied extensively as a molecule of central importance in the long-term culture of T lymphocytes and as a mediator of immune cell activation and interaction mechanisms. Although the potential of IL-2 for therapy of deficiencies in the T cell system was obvious from the date of its discovery, its potential as an anticancer therapeutic was not so clear until studies in which the intraperitoneal (i.p.) or intravenous (i.v.) injection of immune spleen cells, stimulated by incubation with IL-2 in vitro, produced cures in mice carrying syngeneic tumors such as the FBL-3 lymphoma (Cheever et al., 1982; Eberlein et al., 1982). These cures occurred with or without additional injections of IL-2 (Cheever et al., 1982; Donohue et al., 1984a; Eberlein et al., 1982).

Soon after these initial studies, it became possible to utilize recombinant deoxyribonucleic acid technology to produce human and mouse recombinant IL-2s (rIL-2s) (Devos et al., 1983; Taniguchi et al., 1983; Fujita et al., 1983). Although several different expression systems have been developed, a majority of preclinical studies require quantities of rIL-2 generated at industrial levels. This, coupled with the facts that (*a*) the structure of IL-2s and, therefore, by assumption, their functions appear to be reasonably conserved from mouse to man (Taniguchi et al., 1983), (*b*) rIL-2s generated in bacterial expression systems compare identically with IL-2 from multiple lymphoid preparations (Thurman et al., 1986), and (*c*) the industrial development of rIL-2 has coincided with the availability of the recombinant form of the molecule for clinical studies, provides the rationale for this review which focuses on preclinical studies with highly purified, human rIL-2 produced in *Escherichia coli*.

The importance of dose, route, schedule, tumor type, tumor burden, rIL-2-stimulated lymphoid cells (immune versus nonimmune), and combinations of rIL-2 with other lymphokines and xenobiotics has been evaluated in experimental cancer research, but, unlike the historical flow of research in immunology, studies of rIL-2 effects in animal models of infectious diseases have followed, rather than preceded, studies in cancer. Thus, although only a limited amount of literature exists, very recent data concerning the therapeutic uses of rIL-2 for infectious diseases suggest that there may be some important differences in the pharmacology and toxicology of this lymphokine when compared to its properties as an antitumor agent. It is, therefore, important to review the work done during the past 5 years using rIL-2 as a therapeutic for both neoplastic and infectious diseases with the idea that such an overview will lead to a finer appreciation of underlying promises for and problems with this new biological drug. For this reason, information from the recent preclinical literature has been selected for comparison and critical discussion.

As with other proteinaceous "drugs" the rIL-2 molecule presents a host of challenges to the experimental pharmacologist. Native human IL-2 is a core-type glycoprotein with about 10% of its composition being carbohydrate. The protein portion is comprised of 133 amino acids and weighs about 15,000 daltons. There is some heterogeneity in molecular size, probably due to microheterogeneity of carbohydrates, but a key feature is that IL-2 is about 100 times larger than conventional chemical drugs. With IL-2's size and its role as an immune system modulator come increased complexities of structure-function relationships and pharmacokinetics.

The primary structure of human (and mouse) native IL-2 consists of a relatively large proportion of hydrophobic amino acids which is common for proteins that must interact with cell surface receptors. However, the rIL-2 counterpart, although maintaining predominant primary, secondary, and tertiary structural features, also lacks carbohydrate moieties. This most likely adds to the hydrophobic nature of the molecule necessitating formulation in albumin or detergent(s) to maintain solubility.

The secondary structure of IL-2 has been evaluated (Cohen et al., 1986) and, unlike globular proteins, the lymphokine has a large surface to volume ratio consisting of a predominance of α-helical regions which make up about 50% of the molecule in either a classical 4-helix or less common 5-helix bundle. The tertiary structure of rIL-2 has also been discerned by low resolution (5.5 Å) x-ray crystallography (Brandhuber et al., 1987), and results show an overall cup-like structure with dimensions of 40 × 40 × 20 Å which probably bridges two IL-2 receptors on the lymphocyte surface by contact through helices near the amino terminus. Quite interestingly, the molecule has three cysteines and folds during posttranslational events such that the proper disulfide is formed across cysteines 58 and 105, leaving a free sulfhydryl at

position 125 in the linear sequence. Because the recombinant molecule is produced by fermentation in *E. coli* rendering a rIL-2 that is not soluble or properly folded, extraction of the recombinant protein is followed during manufacturing by a folding and disulfide bond-forming process (reviewed by Mark et al., 1987). This tends to allow improper disulfide bonding; however, through a clever application of techniques of site-directed mutagenesis, Wang et al. (1984) substituted a serine for cysteine 125 which is not required for biological activity, thereby leading to a fully active rIL-2 "mutein."

Because rIL-2 is a protein, conventional techniques for (extraction and) assay of drug and/or metabolites, such as high pressure liquid chromatography, are not practical. Thus, both native human rIL-2 and the mutein form are followed during pharmacological studies by enzyme-linked immunosorbent assays and/or bioassays. The latter involve incubating samples containing rIL-2 with lymphocytes (such as the mouse HT-2 cell line, which have proliferation-dependent, highly specific IL-2 receptors) in the presence of [^{3}H]thymidine for several days, after which proliferation is evaluated. Such biological assays are plagued by an inability to differentiate possible metabolites. Thus, the enzyme-linked immunosorbent assay will detect inactive rIL-2, and bioassays will detect partially active forms, including any active metabolites of rIL-2. In fact, the metabolism component in studies of the adsorption, distribution, metabolism, and elimination of protein drugs is an as yet undeveloped, but fundamentally important, area for further studies.

By far the greatest challenge to understanding rIL-2 as a therapeutic is the fact that it is a biological response modifier. This means that, unlike conventional chemical drugs that often directly affect targets of disease such as infectious microbes or malignant cells, rIL-2 depends upon the host's response as the "drug." For this reason, an appreciation of the pharmacodynamics of IL-2 is essential prior to discussion of its pharmacokinetics and therapeutic properties.

II. Pharmacodynamics of rIL-2

A. Effects on the Immune System

1. Effects of rIL-2 on ontogeny and development of immune responses. The normal immune system is unable to respond to immunogens encountered during early states of development (Billingham, et al., 1953). The time course and extent of this unresponsiveness varies from fetal stages to early neonatal periods depending upon animal species. This phenomenon, known as "immunological tolerance" (reviewed by Nossal, 1983), appears in newborn mice to be at least partially due to a deficit in IL-2 production (Ishizaka and Stutman, 1983). Thus, the susceptibility of newborn mice to experimental induction of humoral immune tolerance of non-self-antigens is reversed by exogenous rIL-2 administration (Malkovsky et al., 1985), and newborn mice injected with semiallogeneic cells (to effect tolerance) then treated with rIL-2, reject skin transplants as quickly as controls (Malkovsky and Medawar 1984).

Although an effort to corroborate this finding using another species, namely, the newborn chicken, failed (Tempelis et al., 1988), the attempt points out that species variability in both the time to onset of immune competence and basic immune system differences between species makes all-encompassing conclusions difficult. On the other hand, the chicken does provide a model for spontaneous autoimmune thyroiditis in an obese strain, which also overproduces IL-2 (Schauenstein et al., 1985), and this model brings to question the role of IL-2 in continuation of tolerance during adult life. More studies are clearly needed to probe this possibility as well as the extent to which IL-2 may be involved in autoimmune processes.

When a therapeutic dose of rIL-2 is first administered to an animal or human, one of the initial responses is a pronounced peripheral blood leukopenia (e.g., Lotze et al., 1986). Preclinical data suggest that this initial decrease in peripheral blood leukocytes may be due to margination of cells to parenchymal organs, most notably to the lung and liver (Talmadge et al., 1987), rather than to any type of cell destruction. In fact, several pieces of clinical and preclinical evidence have indicated that IL-2 may actually play a role in natural hematopoiesis. For example, rIL-2 has been found to stimulate bone marrow cell mitosis in rodents in vivo (Gearing et al., 1986), and Estrov et al. (1987) reported that rIL-2 stimulates granulopoiesis in human bone marrow. Although a systematic study of the direct or indirect bone marrow-stimulating activities of rIL-2 over the course of weeks has not been reported to date, it would not be surprising to find that such a phenomenon could occur because accelerated recovery from myelosuppression and prolonged survival following lethal doses of γ-irradiation or cyclophosphamide treatments has been observed with lymphokines (Neta et al., 1986). Furthermore, Conlon et al. (1985) showed a striking ability of exogenously administered rIL-2 to overcome both cyclophosphamide and glucocorticoid hormone-induced immune responsiveness when given to mice 48 h after the suppressive agent. Similar rIL-2-induced reversals are seen using cyclosporin A in studies of contact sensitivity and antibody production in mice (Xue et al., 1986), and Medawar's group has shown that T cell depletion of allogeneic bone marrow prevents the accelerated graft-versus-host disease they see with rIL-2 administration in transplantation studies (Malkovsky et al., 1986).

Although these observations point to a role for IL-2 stimulating myelopoiesis, it is not clear whether the observed effects are a direct consequence of IL-2-stem cell interactions or a secondary effect, such as T cell production of colony-stimulating factors (Metcalf, 1986). Such findings along with the discovery of IL-2 receptors

on human B cells (Waldmann et al., 1984), however, led several groups to study rIL-2 adjuvant-like effects on the IgG and IgM antibody-producing capabilities of genetically low-responding mice. One group found that rIL-2 administration allowed B10.BR mice to overcome *Ir* gene-controlled low responsiveness to myoglobin immunogen, irrespective of immunoglobulin class (Kawamura et al., 1985), whereas another group found a polyclonal stimulation of IgM but not IgG antibody (Weyand et al., 1986). Because concomitant rIL-2 administration with myoglobin did not result in enhanced antibody production in high-responder B10.DR mice, these studies suggest a pivotal role for IL-2 in primary immunity to antigens whose recognition requires participation of molecules from the host's major histocompatibility complex.

At the end of the developmental process is the well-documented decline in immune system function with age. T lymphocytes appear more sensitive to the aging process and display earlier, more severe reductions in activity than B cells (Makinodan and Kay, 1980). Thoman and Weigle (1981, 1985) showed that the capacity to synthesize IL-2 declines with age in mice, although the ability of aged mouse T cells to respond in vitro to IL-2 appears unimpaired. While initial studies were performed with native IL-2 of variable purity, this observation was extended to show that rIL-2 administration enhances the immune response capabilities of aged mice. Similar types of studies in young adult mice also indicated modest but significant increases in both natural killer (NK) cell and cytolytic T lymphocyte (CTL) generation as a result of IL-2 injections (Conlon et al., 1982; Hefeneider et al., 1983).

2. *Cytolytic cell-activating functions.* In addition to serving as a growth factor for T lymphocytes, two additional biological activities of rIL-2 suggest its potential value as an anticancer therapeutic. The first is its ability to activate lymphoid cells to perform cytolytic functions in vitro. This activity can occur independently from the immune status of the lymphocyte population. Thus, in vitro studies during the past 5 years have shown that rIL-2 not only induces the proliferation of antigen-dependent (immune) T lymphocytes and their activation to CTLs but also induces growth and activation of antigen-independent NK (Hefeneider et al., 1983; Handa et al., 1983) and lymphokine-activated killer (LAK) cells (Mule et al., 1984; Rosenstein et al., 1984).

Several important in vitro distinctions are made between these three types of cytolytic lymphocytes. The major differences are in the patterns of serologically defined, cell surface antigens and in the range and specificity of target cells that they kill. In the human, LAK cells are serologically similar to CTLs in that they are both positive for OKT3 and OKT8 (cytolytic/suppressor cell) surface antigens and are devoid of surface markers such as OKT4 (helper/inducer antigen) and OKM1 and Leu-7 which are NK cell markers (Grimm and Rosenberg, 1984). LAK cell populations are further enriched for antigens that define T cell subsets OKT9 and OKT10, for the TAC antigen which is a component of the IL-2 receptor, and histocompatibility markers of the HLA-DR phenotype. Similarly, for the mouse, Rosenstein et al. (1984) showed that LAK activity-expressing cells express conventional CTL phenotypic antigens on their surfaces (e.g., Thy-1$^+$, Lyt-1–2$^+$) but do not express functional antigen or Fc receptors and thus appear to be distinct from CTLs as well. Very recent findings of F4/80, a monocyte/macrophage cell surface antigen, expressed on the same, rIL-2 expanded, L3T4-positive cell population (C. Johnson, personal communication), point to the difficulties faced when trying to relate serologically defined cell surface markers with functional characteristics.

Human CTLs have antigen-binding receptors, using in vitro function as a mechanism for defining cell subsets. CTLs also form rosettes with sheep erythrocytes (and are correspondingly seropositive for the OKT11 rosette receptor), whereas LAK and NK cells do not express these receptors and attendant functions. LAK cells can be further differentiated from CTLs in that the latter have Fc receptors and are "smaller" than LAK cells (Grimm and Rosenberg, 1984). In the mouse, too, NK and LAK cells are differentiated from each other by their range of tumor cell target specificities and the rates at which they lyse those targets in vitro. Unlike NK cells, LAK cells can lyse targets from a wide range of fresh, solid tumors of both human and mouse origin (Grimm et al., 1983a,b, Grimm and Rosenberg, 1984).

While it is clear that rIL-2 can augment cytolytic cell activities ex vivo, it is not clear to what extent this happens in vivo. Because investigators believe that NK and LAK cells are intricately involved in antitumor responses (e.g., Talmadge, 1985), as well as in mediating toxic effects of rIL-2 (see section III and Gately et al., 1988), it is important to understand the potential contributions of these cells in pharmacological terms.

Significant augmentation of splenic, alveolar, hepatic, and systemic lymphocyte NK activity is observed after a single moderate dose (625 IU) of rIL-2 (Talmadge et al., 1985). Whereas this dose is optimal for augmenting lung and liver NK cells, doses a log unit higher are optimal for augmenting NK cells of the spleen and blood, suggesting that lung and liver are more responsive. In addition, daily high-dose (6,250 to 31,250 IU) bolus injections for greater than 4 to 5 days induce a total systemic NK cell hyporesponsiveness. This phenomenon could be due to "exhausted" NK cells, a result of a negative feedback or down-regulation mechanism, or the result of extensive NK cell margination to nonstudied anatomical sites. In any case, one could interpret this finding as a toxic response, and such a response may be more general for cytokines than is currently appreciated.

Just as NK cells appear to exhibit an organ-associated

dose response, it is important for therapeutic purposes to understand which/where lymphocytes can be converted to LAK by rIL-2 treatments in vivo. The potential for a lymphoid cell population to be stimulated in vitro to express LAK activity is the operational definition of a "LAK precursor." Cells with this potential are much more widely distributed throughout the body than are NK cells. For example, human thoracic duct lymphocytes are devoid of NK activity but develop into LAK cells when incubated with rIL-2 (Andriole et al., 1985). In the mouse, LAK cell precursors appear to be devoid of all T cell markers characteristic of CTL precursors (reviewed by Grimm and Rosenberg, 1984), but they do possess the asialo-G_{M1} surface antigen (Lafreniere and Rosenberg, 1985a). Also in the mouse, LAK precursors represent about 1 in every 5000 normal splenocytes, and upon incubation with rIL-2, these cells express Thy-1 and enter the more mature T cell lineage of the animal. This makes one wonder about the ontogenic origins of LAK cells and, in fact, studies by Damle et al. (1986) indicate that a host of lymphocyte subpopulations (including B cells) can exhibit cytolytic activities commonly ascribed to LAK "cells." These authors propose that LAK is an activity rather than a discrete cell type.

The broad distribution of LAK precursors in lymphoid tissues and tumors in both man and mouse (Yron et al., 1980; Vose, 1982; Kedar et al., 1982) coupled with the fact that LAK cell activities can be generated from splenocytes of mice with combined immunodeficiencies in both B and T cell systems (Andriole et al., 1985) suggest that they may play important physiological roles in immune surveillance. In fact, studies in these immune-compromised animals show poor correlations between NK or CTL activity and tumor growth control (Fodstad et al., 1984). Thus, if tumor growth can be affected by cytolytic cells, by process of elimination these cells must have LAK activity.

The problem with this logic is that the naturally occurring LAK cell has been elusive. Thus, only circumstantial evidence for the natural formation of LAK in vivo is available for example, from studies showing that cortisone acetate treatment of animals does not affect the ability to generate LAK cells from isolated splenocytes but does reduce the absolute number of LAK precursors and, by inference, therefore, restricts LAK formation in vivo (Papa et al., 1986a). Further suggestive evidence correlating naturally occurring LAK cells and antitumor responses comes from studies showing that, whereas five of six methylcholanthrene-induced sarcomas of B10 mice can be completely growth inhibited if rIL-2 treatments are carried out early during the tumor latency period, the sixth, rIL-2-insensitive tumor was also the only one that was resistant to the cytolytic effects of LAK cells in vitro (Indrova et al., 1986).

The search for native LAK cells in rIL-2-treated animals has been led by Rosenberg et al. (1985a) who showed that mice given high-dose rIL-2 (33,000 to 66,000 IU) divided into three daily doses for 5 days, i.p.) had some LAK activity in subsequently isolated, organ-associated lymphocytes. Although similar results were obtained by Thompson et al. (1986) with splenocytes and mesenteric lymphocytes, other investigators have been unable to show that LAK cells can be generated in vivo by rIL-2 treatments. No one has been able to isolate LAK activity from the peripheral blood of animals treated in vivo with rIL-2 alone, perhaps because LAK cells, while they do not adhere to nylon wool (as do macrophages), are "sticky" and, when administered i.v., they marginate and adhere in vascular beds where they possess a short (approximately 40-h) half-life (Ettinghausen et al., 1985). These same cells do not appear to demarginate.

Finally, while discussing the biological relevance of cytolytic activities measured in vitro, the phenomenon of antibody-dependent cellular (macrophage-mediated) cytotoxicity, while also an unproven in vivo mechanism, is well described for tumor targets in vitro. And rIL-2 appears to augment this process in vitro (Ralph et al., 1988). Whether this activity is a result of increased Fc-receptor production or increased cellular cytolytic activities as a result of rIL-2 treatment is an important, but as yet unstudied, area for further investigation.

3. Lymphoid cell-recruiting functions. In addition to its potential to act as a T cell growth factor in vitro and to activate leukocytes in areas of inflammation/immune reaction, a third major in vivo biological activity of rIL-2 that suggests its potential utility as a therapeutic agent is its ability to recruit other lymphoid cells to sites of malignancy and/or infection. The basic but controversial tenet underlying the importance of this activity in relation to rIL-2 therapy is that the immune system is continuously monitoring "normal" cell/organ homeostasis and can detect and respond to invasion not only from without (e.g., by microorganisms) but also from within. Thus, as reviewed by Laroye (1973), defects in normal immune surveillance mechanisms may allow neoplastic cell growth. Implicit in any surveillance mechanism is the concept that the host uses cytokines as attractants to help recruit immune cells into neoplastic or inflammatory sites where they can perform cytolytic functions.

Early in vivo studies in which lymphocytes stimulated by rIL-2 in vitro were administered to tumor-bearing animals led to varying conclusions with regard to the cell-recruiting activities of rIL-2. The key question was whether the same cells responding to rIL-2 in vitro, when administered to animals, also functioned in vivo or whether new lymphoid cells are actually recruited to destroy cancer cells. For example, Cheever et al. (1984a,b) concluded that T cells expanded by incubation with rIL-2 in vitro were the major mediators of tumor rejection after transplanting those cells into congenic mice. However, Forni et al. (1985) concluded that local, rIL-2-dependent clonal expansion was not required for

antitumor effects because irradiated immune lymphocytes incubated with rIL-2 and injected with tumor cells were as effective as nonirradiated cells in causing tumor implant rejections, apparently facilitated by lymphocytic infiltrates. Based on this latter observation, the importance of recruitment (or at least involvement) of other lymphoid cells in vivo was convincingly demonstrated when tumor destruction by local rIL-2 treatments of subcutaneous (s.c.) cancer cells mixed with immune cells was prevented by prior sublethal whole-body irradiation (Forni et al., 1985). As provocative as these findings are, the actual role of rIL-2 as a direct recruitment agent or protein attractant remains unproven, as does the relative impact of other factors on antitumor responses.

What has become clear is that many types of lymphoid cells can be expected to respond to rIL-2 treatments by proliferation, recruitment to sites of immune responses, and, importantly, the production of other lymphokines (Handa et al., 1983). For example, following T cell activation, it may be that the release of γ-interferon (IFN-γ) at tumor sites also recruits NK cells (Henney et al., 1981) and perhaps cytolytic macrophage. In addition to these types of effects, one potentially important indirect activity of rIL-2 may be its activation of leukocytes to release tumor necrosis factor (TNF) and/or IFN-γ, both of which have been reported to alter major histocompatibility complex antigen expression on lymphocytes and tumor cells (e.g., Pfizenmaier et al., 1987) and both of which may contribute to toxicity (section III).

4. rIL-2 effects on other subcompartments of the immune system. a. THE IL-2 RECEPTOR COMPARTMENT. As with many other cell surface receptor systems, the IL-2 receptor regulates T cell growth and differentiation. Investigations into the mechanism of signal transduction by the IL-2 receptor indicate that the pathways which utilize increased intracellular calcium, stimulate phosphatidylinositol hydrolysis, and activate protein kinase C are not required (Kozumbo et al., 1987; Mills et al., 1988; Valge et al. 1988). However, as with numerous other polypeptide growth factors, IL-2 induces activation of a tyrosine protein kinase (Saltzman et al. 1988). After binding IL-2, three parameters suffice to initiate entry of the T cell into the cell cycle: IL-2 receptor density, IL-2 concentration, and the duration of interaction between the two. As a result, a critical threshold signal generates an all-or-none quantal entry into cell division (Cantrell and Smith, 1984). Transferred to the in vivo situation, this means that control of the number and expression of IL-2 receptors is not only regulated by IL-2 concentration but is also the central mechanism for either immune enhancement or immune suppression. Exogenous administration of rIL-2 could induce the former and removal of IL-2 receptor-bearing cells might induce the latter. Modulation studies (in which one treats with rIL-2 or with antibody to the IL-2 binding or TAC portion of the receptor, with resulting internalization of the receptor complex) have implicated the IL-2 receptor in myelopoiesis (Burdach et al., 1987), in generation of cells expressing T cell receptors (Jenkinson et al., 1987), and in the pathogenesis of uncontrolled growth of adult T cell leukemia (Waldmann, 1988). What has come out of these studies is a keener appreciation for IL-2's role in immune homeostasis, and one of the most powerful tools for deeper understanding of this role may be through the use of IL-2, IL-2 receptor, and combined IL-2/IL-2*R* transgenic mice (Ishida et al., 1989a,b; Nishi et al., 1988).

There are two affinity classes of IL-2 receptors; about 15% of pM affinity and the remaining bind IL-2 in the nM range (reviewed by Waldmann, 1986). T cells, B cells, and macrophage/monocytes express both forms of IL-2 receptor. While the high-affinity receptor appears to mediate the key physiological response to rIL-2, one of the more fascinating aspects of the therapeutic use of rIL-2 is the finding of soluble, low-affinity IL-2 receptors [which can still bind rIL-2 (Rubin et al., 1986)] in the circulation of humans and animals during rIL-2 therapy (Osawa et al., 1986). Release of soluble IL-2 receptors has been described for activated human lymphoid cells in vitro (Rubin et al., 1985) as well as in patients with immune system disorders such as systemic lupus erythematosus and rheumatoid arthritis (Semenzato et al., 1988). This phenomenon of cells releasing soluble, circulating, receptors in response to therapeutic proteins is not unique to this paracrine hormone, but the phenomenon requires much closer evaluation than it has been given to date. For IL-2, it may represent a mechanism by which lymphokine concentration is controlled locally in areas of inflammation. Release of soluble IL-2 receptors may be one of a myriad of immune suppression mechanisms, and it clearly suggests that careful regulation of IL-2 concentrations in vivo is crucial in immune system homeostasis.

The recent findings that the high-affinity IL-2 receptor complex is expressed preferentially on antigen-activated T cells (i.e., most unstimulated, resting T cells have little detectable IL-2 receptors) and that the IL-2-binding p55 β-chain (or TAC portion) of the IL-2 receptor is expressed at high levels in certain forms of aplastic anemia and adult T cell leukemia (Waldmann, 1988) have led to several new approaches which appear to suppress experimental autoimmune disorders such as autoimmune diabetes mellitus and systemic lupus erythematosus (Kirkman et al., 1985; Kelley et al., 1988).

Two of these approaches involve the selective removal of IL-2 receptor-bearing T cells either (*a*) by the use of antibodies reactive with TAC which, in addition to preventing experimental autoimmune syndromes noted above also specifically prevent cardiac allograft rejection, or (*b*) by the use of a genetically engineered, covalent-complex of rIL-2 and diphtheria toxin (Williams et al., 1987; Bacha et al., 1988), a form of "hormonotoxin" that

can kill T cells involved in graft rejection. These approaches provide the impetus for further development of novel therapeutic agents such as monoclonal antibodies to the p75 α-chain of the rIL-2 receptor and alternative rIL-2-toxin conjugates.

b. THE SUPPRESSOR CELL/FACTOR COMPARTMENT OF rIL-2. While it is clear that rIL-2 activates CTLs to cytolytic activities in vitro, and there is evidence for similar activity in vivo, the role of rIL-2 in regulating mechanisms of self-tolerance or even fetal tolerance (e.g., Tezabwala et al., 1989) is not at all clear. While it is difficult to imagine a role for rIL-2 in clonal deletion or clonal anergy (Nossal, 1989), which is one possible mechanism(s) of self-tolerance, it is not difficult to imagine rIL-2 playing an active role in immunosuppression. Serologically, it is possible to classify subsets of T lymphocytes by their expression of surface antigens derived from the CD8 gene complex. These cells are then defined functionally in vitro by their ability to inhibit the development and/or continuation of an antibody response. The cells that actually suppress the response are called T suppressor (Ts) cells. Ts cells are initially activated by a cell type that has helper activity called a T suppressor-inducer or cognate Ts cell.

There appear to be at least two distinct, antigen-driven mechanisms for generating Ts cells, one major histocompatibility complex restricted and one antigen-only driven (Asano and Tada, 1989). A key dilemma in the immune suppression field is whether or not T cells exist that can down-regulate on-going cytolytic T cell function; such cells may be differentiated from those that inhibit a humoral immune responses by calling the former T suppressor-effector cells. The problem is that in vitro studies indicate that high concentrations of murine, rat, or human rIL-2 generate T suppressor-effector cells that are, indeed, inhibitory to the development of an in vitro alloantigen-specific mixed lymphocyte culture response (Nomi et al., 1984; Ting et al., 1984), but the data suggest that the alloantigen-specific T suppressor-effector cell may also be an alloreactive cytolytic T cell (Schwartz et al., 1982). Thus, a less teleologically pleasing, rIL-2-inducible, suppressor mechanism could be proposed such as activation of allogeneic "veto" suppressor cells which actually are thought to kill cytolytic T cells (Miller, 1980).

While it appears that macrophages also have suppressor-like activity when evaluated in a myeloma cell-spleen cell cytolysis assay (Ye et al., 1984a,b), the isolation of a T cell that inhibits the activities of non-T cytolytic cells has also not been reported. It is possible that unresponsiveness seen with high doses of rIL-2 in in vitro tumor cell cytolysis is a function of the release either of a suppressive factor (Hardt et al., 1981) or simply a function of rIL-2's overwhelming the ability of the cytolytic T cell to mount an in vitro response, perhaps through antigenic receptor down-regulation or cell hypoactivation. Clearly, much more work needs to be done, and the assumption that suppression is a constitutive, dynamic process in vivo leaves room for a myriad of potentially testable hypotheses about how IL-2 may fit in.

B. Vascular, Wound Healing, and Lymphocyte-Trafficking Effects of rIL-2

Most investigations to date have focused on understanding the molecular and cellular mechansims behind the therapeutic index of rIL-2 with only very recent attention to pharmacoydnamic parameters such as effects on fibroplasia or on macromolecule and cell transport and trafficking. Such parameters are not only important for normal homeostasis but are also very important when considering rIL-2 anticancer efficacy because fibroplasia and the transport of macromolecules and cells into the interstitium of normal tissues (reviewed by Aukland and Nicolaysen, 1970; Hay, 1981; Bert and Pearce, 1984) both differ from the same processes in neoplastic tissue (reviewed by Jain, 1987). In general, solid tumor interstitial space is larger than that found in most normal tissues, has a higher concentration of collagen, higher fluid pressure and flow rate, and lacks well-defined, functioning lymphatic networks. Tumor interstitium also has a higher marcomolecular diffusion rate and larger convection components than normal tissues (Jain, 1987). From this standpoint, rIL-2 flow from vascular to interstitial spaces would be favored in solid tumors, and rIL-2 access to tumor infiltrating lymphocytes might be greater than to normal interstitial lymphocytes except, perhaps, in lymphoid organs. Thus, the infiltration of leukocytes from blood pools or across endothelial barriers into tumor masses may be an important component of successful rIL-2 therapy, whether it happens prior to or in conjunction with that therapy.

Basic clues as to the nature of this importance can be gleaned from studies of the effects of lymphokines on fibroblast migration, replication, and collagen synthesis in vitro (e.g., Wahl and Wahl, 1980). These studies have been extended to show that rIL-2 augments fresh wound-breaking strength and collagen synthesis in a rat model (Barbul et al., 1986). Such results indicate not only that lymphocytes are important in wound healing but also that rIL-2 plays important modulatory roles in complex cascades of growth and tissue organizational events. Similarly, there are discrete physiological mechanisms that regulate lymphocyte trafficking.

Polymorphonuclear leukocytes or neutrophils, lymphocytes, and probably monocytes utilize related receptor systems to recognize tissue-specific vascular endothelial cell determinants during the process of extravasation (Lewinsohn et al., 1987). This process is regulated by lymphocyte surface recognition elements which bind high endothelial venules (HEV) (Butcher, 1986). At least two serologically identified and functionally distinct classes of lymphocyte receptors for HEV exist in the mouse: one mediates the recognition of peripheral lymph

node HEV and the other is in mucosal lymphoid organs of Peyers patches (Butcher et al., 1980; Gallatin et al., 1983). While unproven, it is likely that rIL-2 stimulation of lymphocytes bearing such receptors provokes changes in cell trafficking through HEVs, as well as through flat-walled venules. There also appear to be other mechanisms for this latter form of trafficking (Hall, 1980; Rannie and Donald, 1977), and IL-2 could be involved.

Trafficking mechanisms have important implications when rIL-2 is used in conjunction with exogenously administered lymphoid cells. The migration/lodging of administered lymphocytes in the lung has been noted in several studies (LeFever et al., 1984; Stevens et al., 1982). This phenomenon is not likely due to simple trapping of cells in the first capillary bed encountered after i.v. injection because similar results are seen after carotid artery injections (Weisberger et al., 1952), and pulmonary homing is significantly decreased by heat treatment of the cells (Sprent, 1976). One possible explanation is that many tissue sites of lymphocyte infiltration are associated with lymphatic channels, perhaps in areas of increased HEV receptors for lymphoid elements. In the lung, lymphatics travel with pulmonary veins, arterioles, and bronchi and beneath visceral pleura (Lauweryns, 1971). In the kidney, lymph channels lie beneath the renal capsule and freely communicate with plexi and perirenal fat (Peirce, 1944). These are generally areas in which one finds exogenously administered LAK cells. However, LAK cells are also found in several areas including liver sinusoids which do not contain lymphatic vessels (Ettinghausen et al., 1985), and "hepatic trapping" after LAK cells flow through the lungs cannot be ruled out.

Finally, when considering potential roles of lymphokines in regulating lymphocyte trafficking de novo, the effects (direct or indirect) of rIL-2 on primary mediators of acute inflammatory responses, i.e.,, circulating neutrophils, should not be underestimated. These cells exist in dynamic equilibrium between a freely borne pool and a marginating pool interacting reversibly with the endothelium (Athens et al., 1961). In response to inflammatory stimuli, freely circulating neutrophils adhere tightly to the vascular endothelium, migrate through vessel walls, and move along chemotactic gradients toward the stimulus (Harlan, 1985). During this process, a host of factors are released, and such leukocyte-endothelial cell interactions could well be a central mechanism in rIL-2's cell recruiting activities. For example, this action of rIL-2 could help explain nonimmune mechanisms of tumor destruction (by indirectly mediating tumor vascular bed collapse) and could contribute to dose-limiting toxicities which are described briefly in section III.

III. Toxicity: The Dose-Limiting Characteristics of IL-2

A dose-limiting toxicity of rIL-2 in clinical studies is a capillary network-based "vascular leak syndrome" (VLS) which results in anasarca and multiorgan system dysfunction (Lotze et al., 1986). Although reversible upon termination of rIL-2 therapy, this phenomenon caused Rosenstein et al. (1986) to evaluate VLS in a mouse model in which they found that the degree of leak of radiolabeled albumin into tissues correlates with rIL-2 dose and duration of rIL-2 treatment. While the data may be difficult to interpret because lymphoid organ hyperplasia was not included in evaluating the degree of "leak," it appears that this process also occurs in mice, and the time course of events (days rather than minutes) is similar to that reported in human studies.

Generalized VLS induced by high, repeated doses of rIL-2 is seen reproducibly in other animal model (Rosenstein et al., 1986; Fairman et al., 1987), but a potentially related phenomenon, namely, increased leak of macromolecules across the blood-brain barrier after single, low-dose rIL-2 treatments in a cat model (Ellison et al., 1987) may not be reproducible because these latter studies suffer from technical problem. However, rIL-2 does penetrate the blood-cerebrospinal fluid barrier (Saris et al., 1988), and this may be exaggerated when the barrier is disrupted as with a glioma model (Alexander et al., 1989). This is not to imply that rIL-2 may not affect neuroendocrine mechanisms. A resurgence in studies of the effects of neuroendocrine hormones on immune function led Farrar et al. (1984) to report the stimulation of endorphins by IL-2, and recent studies by Rosenberg's group have shown increased levels of β-endorphin, corticotropin, cortisol, and corticotropin-releasing hormone in patients (Denicoff et al., 1989). In a perhaps related, provocative finding, Tuttle and Boppana (1990) showed that single, low-dose injections of rIL-2 permanently reverses hypertension in the spontaneously hypertensive rat—perhaps as a result of affecting endocrine/immune system imbalance in this model. However, a pulmonary "leak syndrome" remains the major dose-limiting effect of rIL-2 treatment in clinical studies, and the question of a good preclinical model for evaluating this phenomenon is, therefore, of concern as a topic of this review. While the rat does not apparently demonstrate a strong VLS upon rIL-2 administration, at least in the lung, studies in rats given rIL-2 daily for 14 to 16 days yielded hematological, clinical chemical, gross pathological, and histopathological findings similar to those reported in man. Hemolytic anemia, lymphocytosis, neutrophilia, eosinophilia, thrombocytopenia, increased hepatic transaminases, hyperbilirubinemia, hypoalbuminemia, marked lymphocyte infiltration of liver and eye (associated with hepatic necrosis and retinal damage), and splenic extramedullary erythropoiesis and eosinopoiesis were all dose-related findings (Anderson and Hayes, 1989). Of importance in later discussion of rIL-2 pharmacokinetics is the finding that the severity of these toxic effects was increased if the dosage was split into a twice-daily regimen.

The fact that rodents tend to make more of a hepatosplanchnic response in deference to cardiopulmonary response during inflammatory reactions (and apparently during IL-2 treatments) led several investigators to shift focus to an animal model that has more of the human type of (cardiopulmonary) response during systemic inflammation. The sheep provides such a model and, because it also has a uniquely accessible lymph-draining system, cardiopulmonary effects of rIL-2 have been evaluated in a conscious sheep/lung-lymph fistula model (Glauser et al., 1988). This model, as is seen in the clinical setting (Rosenberg et al., 1987), shows that most of the pulmonary VLS effects appear to be secondary to a decreased systemic vascular resistance and an (presumed related) increase in capillary permeability (Jesmok and Gunther, 1989). The time course of these toxic events suggests activation of cascades of pharmacological mediators, which could potentially be inhibited by pharmacological intervention.

Several potential mechanisms could be blocked. For example, the indirect effects of rIL-2 on leukocytes could involve production of oxygen-free radicals or arachidonate metabolites by activated macrophages (Geczy, 1984). Potential rIL-2-associated secretion of vasoactive amines and similar factors by lymphocytes and neutrophils (reviewed by Willoughby, 1973; Sobel and Lagrue, 1980) could be targets for toxicity amelioration. These mediators, if responsible, would likely act directly on endothelial cells by making them "stickier," by facilitating lymphocyte-endothelial cell interactions or by causing a relaxation of gap junctions. Alternatively, based on in vitro models, investigators propose that rIL-2 acts in vivo by the production of cytokines such as TNF and IL-1 which then, along with rIL-2, can activate human lymphocytes to exhibit enhanced adhesion to normal vascular endothelial cells with an ultimate effect being endothelial lysis (Tracey et al., 1986; Damle et al., 1987; Aronson et al., 1988). While this is an unlikely event in vivo, the controversy concerning whether cell damage/lysis occurs or gap junctions merely open needs resolving with an eye toward improving rIL-2's therapeutic index. Successful pharmacological approaches to ameliorating VLS should help resolve this issue.

Most of the cumulative toxicities of rIL-2 are remarkably similar to the well-documented hemodynamic changes in septic shock (Thijs et al., 1988), including potential activation of complement components via TNF or IL-1 secretion (Okusawa et al., 1988). The sequence of events in septic shock is also similar to the sequence of events in rIL-2 toxicity, but a key question which remains unresolved is whether the rIL-2-induced leak reflects a generalized, systemic inflammatory reaction or is, in some way, a unique property of high-dose rIL-2 therapy.

This is an important question. If rIL-2 induces generalized inflammation, then secondary effects such as complement activation and eliciting of acute phase proteins would tie rIL-2 toxicity perhaps too closely with efficacy to hope for improvements in therapeutic index by pharmacological intervention.

One approach that could help clarify the similarities between rIL-2 toxicities and septic shock syndrome would be to unravel the time course of events in VLS. Normal migration of lymphocytes across HEVs appears to occur without significant associated fluid loss (Schoefl, 1972). In acute inflammatory responses, increased vascular permeability occurs with a fluid leak phase that is temporally separated from and often completed by the time of onset of leukocyte emigration from vessels (Hurley, 1963, 1984). Investigations into the temporal relationship between rIL-2-induced VLS and leukocyte migration may lead to ways to prevent leukocyte adhesion, especially if cells migrate before fluid leak.

IV. Efficacy: rIL-2 in Cancer Therapy

A. Immunological Parameters

1. The immune response and tumors. The concept of manipulating the immune response as part of anticancer therapy is a historically venerable one that dates back to the 1890s (Herecourt and Richet, 1895). In addition to assuming immune surveillance (Laroye, 1973), the basic premise underlying immunotherapy for cancer is that malignant cells contain specific antigens that differentiate them from normal tissue and that the immune response of the tumor-bearing host recognizes these antigens and could make a more effective (rejection) reaction to them if given some outside help. Approaches to specific immunotherapy, whether active (by vaccination) or adoptive, require that there be tumor-related antigens. All other immune therapies rely on the concept that, even if the immune response cannot be directed specifically against the tumor, the process of eliciting an irrelevant immune reaction, an inflammatory response, or simply lymphoid cell recruitment to the neoplasm/vicinity may lead to tumor destruction via innocent bystander phenomena.

Fundamental aspects of tumor cell surface antigenicity, mechanisms of tumor escape from the immune response, and alteration of tumor surface antigens have been reviewed previously (Jelsema et al., 1981). Virtually all experimentally induced neoplasms have tumor-related or tumor-specific transplantation antigens associated with their cell surfaces that can be detected both in vitro and in vivo (Klein, 1966), but one of the most controversial features of studies of progressive growth of immunogenic tumors of mice is the problem of discerning the relative roles of host immunity versus tumor cell heterogeneity in the mechanism(s) of escape from immune rejection. There are two clearly opposing views. First, there is evidence that mice develop Ts cells that down-regulate antitumor responses before these re-

sponses can develop enough to cause tumor rejection (North, 1982, 1985).

On the other hand, escape of immunogenic tumors from immune recognition has also been attributed to phenotypic (antigenic) heterogeneity which appears to be an inherent characteristic of many tumors (Nowell, 1976). Investigators favoring this latter line rely on in vitro data which suggests that T cells exert strong selective pressure leading to the outgrowth of cells expressing fewer or different tumor-associated antigens (Urban et al., 1982). Antibodies have also been implicated, at least in the process of tumor progression (Winkelhake et al., 1979). However, the loss of susceptibility to lysis by CTLs in vitro or the loss of surface antigens during progressive growth does not imply that later tumor cell populations have the capacity to escape immune rejection in vivo (Fahey and Hines, 1987). Current evidence favoring tumor antigen recognition by T cells is strongly circumstantial and, in fact, there is really no formal evidence that conclusively links CTLs to tumor destruction in vivo (Robins and Baldwin, 1985). More than likely, the answer for human cancer lies in some combination of humoral and cellular immunity and, if the patient can mount an immune response against his or her tumor, immunotherapy will be efficacious.

While the presence of tumor-associated antigens on human neoplasms is not established, experimental animal tumor models still provide the ideal testing ground for evaluating rIL-2's role as a component of the immunotherapy strategy for cancer. Of primary concern, however, is the question of the degree of tumor immunogenicity in relation to rIL-2's potential therapeutic index and other pharmacological parameters with possible human clinical significance. Because most animal tumors can be described as immunogenic to one extent or another, one would expect that when low, nontoxic doses of rIL-2 provide adequate antitumor responses investigators may be working with highly immunogenic tumors. In addition, one would not expect successful rIL-2 therapy when rodent tumors, responsive in immunologically intact animals, are evaluated in immune-compromised animals (e.g., the congenitally athymic, nude mouse). Similarly, when the question of rIL-2 efficacy is studied in relation to an ongoing host immune response, it would be predictable to find that immunosupportive effects of rIL-2 therapy are hidden.

Masking of potential therapeutic effects is, in fact, what Vaage et al. (1987) found in mice preimmunized for 6 days with the MC2 mammary carcinoma. In these studies, rIL-2 therapeutic effects on new tumors implanted in the opposite flank 6 days after a primary implant could not be distinguished from controls receiving no rIL-2 therapy. Results led the investigators to speculate that, in "strongly immunized" mice, rIL-2 therapeutic efficacy could be obscured by "well developed resistance factors." It is not difficult to take the next step in logic and then conclude that, with highly immunogenic tumors, rIL-2's potential therapeutic efficacy could be easily overestimated.

2. Adoptive immunotherapy of cancer: theoretical considerations. The concept of adoptive immunotherapy with lymphoid cells or with immune sera stemmed from observations of Landsteiner and Chase (1942) who demonstrated that delayed hypersensitivity skin reactions could be transferred successfully via immune cells from immune guinea pigs to nonimmune recipients. Old et al. (1961) later showed that transplantation of syngeneic lymphoid cells from actively immunized mice conferred tumor-specific immunity to the recipients. The fact that antitumor effects of such passively transferred immune cells were abolished by either splenectomy or blockade of the host's mononuclear phagocyte system with colloidal carbon (Alexander et al., 1966) suggested that transferred lymphocytes do not exert their effects alone but rather act as immunostimulants for host defense mechanisms. Thus, the host's immune system "adopts" tumor immunity. A key point is that adoptive immunotherapy requires an active participation by the host's immune response and is not solely the passive transfer of immune cells or serum.

While basic immune cell transfer experiments are generally successful with animal tumors, the major obstacle with passive transfer of immune lymphocytes in man (making the assumption that tumor antigens exist and are limited in number or are specific to tumor types) is the incompatibiity of transplantation antigens. One approach that by-passes these problems, but that has met with inconsistent success, has been the in vitro activation of autologous immune lymphocytes by mitogens with reinfusion into patients (Mazumder et al., 1984a,b). If this approach were extended to the use of autologous nonimmune lymphoid cells, it would be possible to imagine a therapy for individual tumors even if they are not immunogenic.

Rosenberg and colleagues capitalized on this latter concept by incubating murine or human lymphocytes (isolated from autochthonous, tumor-bearing animals/humans) with rIL-2 for subsequent adoptive cellular immunotherapy (Yron et al., 1980; Lotze et al., 1981). Using radiolabeled LAK cells, these investigators also showed that, after adoptive transfer, mouse cells undergo active in vivo proliferation that is dependent upon concomitant administration of rIL-2. Furthermore, when rIL-2 is administered alone, lungs, liver, spleen, kidneys, and blood show expansion of endogenous lymphoid cells, whereas rIL-2 + LAK administration results in additional increased cell numbers and cytolytic activity of lymphocytes isolated from lung, mesenteric nodes, and liver (Ettinghausen et al., 1985).

The conversion of human peripheral blood or murine splenic lymphocytes to LAK cells/activities has decided advantages for therapy because these cells have tumor

cytolytic activity whether or not they have been exposed to the autologous tumor antigens before. And, because the cells are initially isolated from the individual who will receive them after rIL-2 incubations, there are no known problems with histocompatibility antigens. The question of whether human HLA or murine H2 antigen distribution/expression could be altered by in vitro culture, thereby contributing to LAK activity, has not been evaluated.

B. Pharmacological Parameters of the Antitumor Effects of rIL-2

Under normal physiological conditions, rIL-2 is thought to be a paracrine hormone that operates in localized areas of inflammation or immune reaction and is not measurable in the circulation. Therapeutic use of this lymphokine assumes benefit in the amplification of presumed insufficient immune mechanism(s) by administering rIL-2 at pharmacological doses rather than in reconstitution of an abnormal physiological response (as is the case with insulin therapy). Because the host is not normally exposed to high circulating levels of this protein, and because rIL-2 is administered with the intention of converting the host to a biologically responsive "drug," pharmacological characteristics of dose, route, and schedule of administration become perhaps even more important than classically considered as a means of understanding efficacy. These parameters have been best evaluated in immune-competent rodent models and, unless otherwise stated, will be the subject of this section.

1. Pharmacokinetics. Initial pharmacokinetic studies performed with rIL-2 showed that, irrespective of route, T cell growth was augmented when donor T cells were implanted i.p., implying good whole-body bioavailability (Cheever et al., 1985a,b; Donohue and Rosenberg, 1983). In fact, if one evaluates areas under blood clearance curves, the volume of distribution of rIL-2, irrespective of route, for mouse, rat, monkey, and man is about equivalent to the total calculated extravascular space, irrespective of species. Furthermore, clearance profiles show that this protein drug follows classical pharmacokinetic behavior as animal species size increases, namely, the systemic clearance value for rIL-2 is normalized when one compares dose on a mg/m^2 rather than mg/kg basis (J. D. Young, personal communication).

Following i.v. administration to mice and rats, rIL-2 disappears from the circulation with a pharmacokinetic profile of either a two- or three-compartment model depending upon assay sensitivity. The first phase has a half-life of between 4 and 6 min, during which time approximately 75% of the dose disappears from the circulation. The secondary phase has a half-life of between 2 and 4 h, as does the major clearance phase seen with either i.p. or s.c. injections (Donohue and Rosenberg, 1983; Donohue et al., 1984b; Matory et al., 1985).

In initial bioavailability studies, growth of i.p. implanted T cells correlated with duration of rIL-2 serum levels rather than peaks. Because initial bioassays allowed looking only at early time points, it appeared that rIL-2's serum half-life was shortened when administered i.v. compared with when given i.p. or s.c. This is not the case because rIL-2 is only about 50% bioavailable from the peritoneal cavity, but, as the i.p. route is much more convenient for multiple dosing regimens in rodents, a majority of antitumor efficacy studies have used this route of administration.

The kidney is known to play a major role in filtering and metabolizing proteins of less than 50,000 to 60,000 molecular weight (Strober and Waldmann, 1974) and, as expected, the primary organ of rIL-2 clearance is the kidney (Donohue and Rosenberg, 1983). Investigators find no intact (bioassayable) rIL-2 in the urine, and bilateral ureteral ligation, unlike total renal ligation, has little affect on the short half-life, suggesting renal tubular degradation (Donohue et al., 1984b; Koths and Halenbeck, 1985) with a mechanism perhaps similar to the serum clearance, metabolism, and excretion of Bence-Jones proteins (Wochner et al., 1967).

2. Controlled/sustained-release dosage forms of rIL-2. Based on the initial pharmacokinetic data, three separate approaches have been taken to develop controlled or sustained-release dosage forms of rIL-2. In the first case, the lymphokine has been trapped in sustained-release vehicles such as gelatin or a pluronic gel copolymer (Morikawa et al., 1987). In the second case, mini-osmotic pumps have been implanted to provide a continuous infusion dosage form (Nishimura et al., 1986a,b). Finally, rIL-2 has been "polyethylene glycolated" (Katre et al., 1987) and, as is seen with many other small proteins, covalent modification with polyethylene glycol enhances circulatory longevity (Davis et al., 1980; Beauchamp et al., 1983; Abuchowski et al., 1984). In the case of rIL-2, this means extending the half-life of the major phase about 15-fold (from hours to days), presumably by inhibiting renal clearance, and it means marked improvement in potency and antitumor efficacy (Zimmerman et al., 1989a). No matter what method of sustaining blood levels of rIL-2 is used, antitumor efficacy is improved, and peak doses required for the same effects are lowered, implying an improved potency and perhaps an improved therapeutic index, although this latter point remains speculative.

3. The role of dose. Some controversy exists regarding the importance of dose in preclinical studies of rIL-2 efficacy. While most investigators report that repeated injections of low (hundreds of units) doses of rIL-2 fail to reduce tumor growth in syngeneic rodent systems, a few have demonstrated efficacy with low-dose therapy. The latter find that antitumor effects of low-dose rIL-2 are also independent of schedule. To date, it appears that these differences may be attributed to tumor type, to the degree of tumor immunogenicity, and to the route of rIL-2 administration.

For example, while most investigators (Rosenberg et

al., 1985b; Cheever et al., 1982; Forni et al., 1985; Donohue et al., 1984b; Mule et al., 1984) using either i.p., i.v., or s.c. routes for doses that are considered "low" and range from several hundred to several thousand IU saw little efficacy, Vaage et al. (1987) showed significant tumor growth inhibition at 100-IU doses when the rIL-2 was administered s.c. around the tumor in a strongly immunogenic cancer model. Because human tumors are not as likely to be as immunogenic and because metastatic cancer is often not accessible to peritumoral injection, these types of animal models tend to over-optimistically suggest a potent therapeutic index for rIL-2. They certainly suggest that the drug is potent if delivered directly to an immunogenic tumor.

The first evidence that "high-dose" rIL-2 (15,000 to 20,000 IU/day) is more efficacious was obtained by Rosenberg et al. (1985a) who showed that thrice daily injections of doses totaling up to 18,000 IU/day of rIL-2 induced regression of metastases established 10 days prior to initiation of therapy in a methylcholanthrene-induced sarcoma, MCA-105. This model involves injecting 3×10^5 tumor cells i.v., injecting india ink intratracheally 15 days later (tumor nodules then appear white against a black lung background), and counting pulmonary metastases. Results of these studies showed that these high daily doses gave remarkable antitumor effects. What was most provocative about the studies was the additional fact that pulmonary metastases allowed to "take" for 10 days were more amenable to treatment than 3-day-established pulmonary tumors.

Similar results were found in the s.c. MCA-105 sarcoma model in which more established tumors respond with cures of between 15 and 40% of the animals compared to very few cures if treatment is carried out for only a few days after tumor implantation (Rosenberg et al., 1985a). The conclusion after initial interpretation would be that the longer period between tumor implant and onset of therapy allows for building a host immune response, which is then augmented with rIL-2. Such a mechanism could explain why Thompson et al. (1986) found that, when moderate doses of rIL-2 were given to animals bearing the FBL-3 leukemia (8000 IU, daily, i.p.) between 5 and 9 days after tumor implantation, 50% were cured, whereas the same therapy between days 0 and 5 was ineffective.

The relationships between rIL-2 therapeutic dose/schedule and time of host exposure to tumor are complex, and antitumor effects appear to be unique to the animal model under study. For example, a multiphasic dose response is seen with rIL-2 treatment in a spontaneous metastasis model using footpad-implanted B16 melanoma cells, hindlimb amputation after 4 weeks, and lung colony assay at 8 to 10 weeks (Talmadge et al., 1987). The authors' theoretical explanation for the triphasic dose response seen in this study is that low rIL-2 doses activate the T cell system, high doses activate NK/LAK cells/activities, and intermediate doses of rIL-2 (8,000 to 16,000 IU) activate suppressor cytolytic T cells or cause release of macrophage suppressor factors (Hardt et al., 1981). Such an interpretation is consistent with the finding that athymic nude mice exhibit only the high-dose response (Talmadge et al., 1987).

However, at least two alternative hypothetical explanations for the triphasic dose-response curves come to mind. First, it may be that low-dose effects are primarily augmented CTL-type immune responses, intermediate doses could result in overwhelmed tumor antigen-specific immune mechanisms, perhaps altering CTLs to a nonspecific mode (Damle et al., 1986), while high-dose effects could be the result of vascular and/or generalized and nonspecific inflammatory reactions. Second, in models in which such triphasic responses are seen, the time course from therapy to measurement of response is generally well beyond 3 to 4 weeks (enough time for an animal to mount a neutralizing anti-rIL-2 antibody response). Thus, low-dose therapy may be efficacious because there is not enough rIL-2 to elicit significant levels of rIL-2-neutralizing antibodies. High-dose therapy may be efficacious because there is plenty of rIL-2 around such that it "floods" the humoral response; while moderate doses of rIL-2 may not show efficacy because they provide for an optimal humoral response and subsequent "neutralization" of rIL-2 efficacy.

4. Route-related phenomena. Early pharmacokinetic data suggested that when given i.p. or i.v. rIL-2 distributes readily into various bodily compartments (Donohue and Rosenberg, 1983), and few studies have been performed using other routes of administration. However, some basic, supportive information about rIL-2 pharmacology is available from nonstandard route studies. For example, in studies of antigen-specific combination therapy using cyclophosphamide, rIL-2 and an extract of the MCA fibrosarcoma antigens, Nomi et al. (1986) compared direct intrasplenic injections of rIL-2 with i.p. injections. These authors believe that high doses of rIL-2 generate Ts cells and thus did not test very high doses. However, this and a similar study with combination rIL-2 and chemotherapy (Kahan et al., 1986) clearly showed increased inhibition of s.c. tumor growth when rIL-2 was administered intrasplenically. Improved efficacy was attributed to local lymphatic drainage of the s.c. tumor site by the spleen. These results support conclusions from the peritumoral, low-dose studies in that both suggest that, if one is able to direct rIL-2 to specific sites including the tumor and nearby lymphoid organs, improved efficacy should be anticipated.

5. Schedule of administration. The question of how often and how long to administer rIL-2 has been a perplexing one since early in vivo studies. The problem is characterized by a general lack of knowledge of mechanisms involved in rIL-2 pharmacodynamics and by the fact that animal tumors are so fast growing that they

result in death or massive tumor burdens which can overwhelm even the heartiest immune system within a few weeks. Human tumors are much slower growing. This fact added to data from clinical studies using continuous infusion rIL-2 and LAK (West et al., 1986) and preclinical pharmacokinetic data suggesting improved efficacy with controlled-release rIL-2 (Morikawa et al., 1987; Nishimura et al., 1986a,b; Katre et al., 1987) provided a rationale for continuous infusion studies in the 10- to 12-week B16 melanoma model of spontaneous metastasis after hindlimb amputation (Talmadge et al., 1987). In this model, rIL-2 treatment begins after primary amputation, and with continuous rIL-2 administration, significantly greater activity is seen with doses of 35 IU/animal/h than is seen with i.v. daily bolus doses of 10-fold greater or 10-fold lower. A very recent and provocative set of studies by Zimmerman et al. (1989a) showed a tight schedule dependency to the murine antitumor effects of rIL-2 such that they proposed the need for daily "threshold" levels of continuous rIL-2 exposure in order to elicit an optimal host (immune) response to the tumor and a daily "drug holiday" to reduce toxicity.

Thus, in both preclinical and clinical studies, continuous infusion or even thrice daily bolus dosing of rIL-2 appears to provide better efficacy than a maximum tolerated dose (MTD) daily bolus schedule. And, as expected from conclusions drawn so far, daily bolus treatments at MTD for less than 4 weeks in the model described above gives insignificant efficacy, while daily bolus therapy for 4 weeks or greater gives significant reduction in the number of metastases (Talmadge et al., 1987). This latter result speaks strongly against immune response augmentation as the only mechanism of rIL-2 efficacy and coupled with the recent provocative discovery of an apparent separate schedule dependency for rIL-2 efficacy and toxicity (Zimmerman et al., 1989a), suggests that it may be possible to separate efficacy and toxicity by manipulating the scheduling of rIL-2 treatment.

In summary, it is reasonable to conclude at this time that, for slow-growing tumors, continuous presence of rIL-2 for long periods is more efficacious, whereas for rapidly growing immunogenic tumors, high dosages early after tumor challenge give better efficacy. This line of thought would predict that administration of high doses of rIL-2 for brief (pulsed) intensity/duration periods would be most efficacious.

C. Oncological Parameters of the Antitumor Effects of rIL-2

There seem to be no general principles relating tumor type to efficacy results with rIL-2 therapy in animal models. Immunogenicity is one key but is not the sole determinant. In the one investigation reported using a rat model of spontaneously arising, chemically induced carcinoma (colorectal), there was no apparent correlation between delayed tumor growth and immunological parameters (Garzon et al., 1986). It is also of interest that, unlike the common, transplanted, immunogenic rodent tumors, neoplasms that are known to be particularly aggressive (i.e., grow rapidly and metastasize readily from primary s.c. implants) and are difficult to treat with conventional xenobiotic agents [such as the pancreatic carcinoma of the C57B1 mouse, PAN-02 (Corbett et al., 1984)] are also extremely refractory to rIL-2 therapy (personal observations). Thus, in addition to immunogenicity, other factors that have been shown to impact the antitumor effects of rIL-2 include tumor burden, tumor site (e.g., i.p. versus s.c. sites), and metastatic/growth potentials.

1. Relative degrees of tumor immunogenicity. Because rIL-2 augments immune/inflammatory responses from the host, the therapeutic ratio of rIL-2 is very dependent upon the individual host's susceptibility to immune modulation. Thus, while untested, it is possible to imagine that individual strains of animals might be more or less readily affected by rIL-2 than other strains. Similarly, a host/tumor treated with immune system-altering drugs/radiotherapy, etc., would be less responsive to rIL-2 than an uncompromised animal. Add to possible host variability the differences in immunogenicity of rodent tumors, and one finds that the question of determining a rIL-2 therapeutic index becomes very complex.

The classical definition of tumor immunogenicity is based on in vivo parameters, for example, how often can the same tumor be reimplanted into the same animal before being rejected? This is a very rigorous definition because the answer relies on the host's total capacity to reject a tumor graft. Chemically induced neoplasms tend to be more readily recognized and rejected on reimplantation after removing the primary tumor than do spontaneously arising neoplasms. However, even within the former class, it is possible to subclassify tumors into various categories of immunogenicity. For example, Rosenberg's group chose to classify "weakly and nonimmunogenic" sarcomas based on whether tumor growth (progression) is affected by prior vaccination of the animal with viable tumor cells and *Corynibacterium parvum*. In this scheme, if reimplanted tumors grow well after primary amputation and if the tertiary tumor implant grows at the same rate as that at the vaccination site, the tumor is classified as nonimmunogenic (Lafreniere et al., 1985a). Because these tumors vary in their abilities to "escape" immune recognition and/or destruction, for example, through antigenic modulation, the potential for misclassification (especially as nonimmunogenic) exists.

In addition to chemically transformed sarcoma/carcinoma models such as those described above, there are a number of other rodent tumor types that have been utilized for evaluating chemotherapeutic anticancer agents. These tumors retake readily in animals that have had the primary implant removed or cured, and they all generally tend to induce a pronounced immune suppres-

sion after a week or two of growth. By the criteria listed above, these tumors would be listed as weakly immunogenic or even nonimmunogenic, but they all have definable tumor-associated antigens. For immunotherapy purposes, such tumors should be considered moderately immunogenic, and all seem to respond marginally to rIL-2.

An example of this phenomenon is the P815 mastocytoma of DBA/2 mice (Takei et al., 1976) which grows relatively rapidly and disseminates widely with a measurable but transient immune response about 8 to 10 days after i.p. tumor cell administration. The host's immune response appears to slow tumor growth during the first week, but the tumor soon overcomes this effect and continues to grow resulting in host death between 20 and 30 days after cell implantation. Treatment of s.c. P815 tumors with rIL-2 alone results in a measurable but also transient inhibition of tumor growth if therapy is initiated within 1 to 3 days after tumor cell implantation (Winkelhake et al., 1987), suggesting that an adjuvant-like mechanism is involved. However, later growth (2 weeks after cell implantation) and survival are not affected. Similarly, the ultraviolet light-induced, B16 melanoma of C57B1 mice, which contains tumor-associated surface antigens (Winkelhake et al., 1979), induces a measurable NK cell immune suppression after 6 to 7 days of growth (Lala et al., 1985) and responds in the s.c. site only marginally to MTD daily bolus rIL-2 therapy (Winkelhake et al., 1987). The reason(s) for tumor progression, for example, whether tumor-induced suppression occurs or anti-rIL-2 antibodies "neutralize" rIL-2 antitumor effects, etc., have not been evaluated. However, mechanisms are crucial because the human antitumor response often occurs at some time after rIL-2 therapy and may be tied into similar mechanisms(s).

2. Role of tumor burden. One general conclusion that can be derived from nearly every animal model study using immunomodulatory proteins is that repeated administration with therapy initiated during the very early 0- to 3-day "latency" period after s.c. tumor implantation is more effective than identical therapy initiated after the tumor has "taken" (Talmadge and Herberman, 1986; Winkelhake et al., 1987). There can be overriding phenomena such as those related to immunogenicity or anomalies of tumor location that make this conclusion less firm, but early and prolonged treatment regimens are usually superior. For example, Bubenik et al. (1986) found that the methylcholanthrene-induced sarcoma MC11 could be prevented from appearing in 39% of the animals if treatments were given during the first 12 days after tumor cell implantationss. There was, however, no significant antitumor effect if the same 12-day rIL-2 therapy was initiated 1 week after tumor cell implantation.

There are probably several, interrelated reasons why s.c. rodent tumors are especially susceptible to rIL-2 therapy very early after implantation. First, as discussed above, the animal can mount a rigorous immune response during the first weeks, and the magnitude of this response may be boosted by rIL-2 in an adjuvant-like fashion. This boost may then be sufficient for rejecting small tumor burdens especially if tumor "escape" mechanisms, such as the release of soluble tumor antigens, are poorly developed during this early period. Second, it appears to take several days for tumors to establish a vascular bed prior to rapid growth (Zeidman, 1967), and rIL-2 interference with angiogenesis and/or endothelial cell functions could provide antitumor/antimetastatic effects during this latency period. The importance of these latter phenomena are only now being recognized, and many more studies are needed to understand and perhaps to take advantage of potential antineovascular effects of rIL-2.

3. Role of tumor location. a. PERITONEAL TUMOR MODELS (EXTENSION OF LIFETIME STUDIES). The use of the peritoneal cavity as a compartment for studies of antitumor efficacy is based on its well-defined limits, ease of accessibility for treatment regimens, presence of relevant effector cells (NK and macrophage), and the fact that the effector cells may have ready access to tumor cells. Practically, i.p. models can also serve to some extent as representatives of the minimal residual disease that often then recurs in the clinical setting. The peritoneum is thus an ideal setting for evaluating cytotoxic compounds and has been an integral part of oncology for many years (Markman, 1985, 1987). However, it probably has limited usefulness for lymphokine evaluation for two important reasons: First, despite rIL-2's ability to transiently augment NK cell activity in the peritoneum (Talmadge, 1985), the treatment of i.p. tumors with rIL-2 alone has met with little meaningful success irrespective of dose or schedule. When efficacy is found in i.p. tumor models, it is after early initiation of therapy, with low tumor burdens, and rarely are cures seen (Salup et al., 1985; Winkelhake et al., 1987). The reason for this go beyond the findings of NK cell inactivation during the period of tumor growth (Lala et al., 1985) and depend upon the relationships among tumor growth rate, immunogenicity, and the uniqueness of the peritoneal cavity in these regards.

Some tumors grow and metastasize locally with greater ease from the peritoneum than from s.c. sites. Such rapid growth rate precludes time for adequate host immune response even for highly immunogenic tumors such as the Meth-A sarcoma or EL-4 lymphoma. Thus, these tumors are refractory to rIL-2 therapy when growing in the peritoneal cavity, whereas they show significant responses to rIL-2 treatments when growing in a s.c. site where time-to-death is also much extended (Winkelhake et al., 1987). Similarly, the i.p. models of moderately immunogenic, but rapidly growing, murine tumors such as the B16 melanoma, L1210 leukemia, and P815 mastocytoma are not significantly affected by i.p. therapy

with rIL-2 alone, even though their growth in s.c. sites is inhibited by the same dose regimens (Winkelhake et al., 1987).

Conversely, tumors that are lethal within 4 to 8 weeks tend to show more significant responses in i.p. models. For example, the renal cell carcinoma of BALB/c mice, Renca, described originally in a subrenal capsule model by Murphy and Hrushesky (1973), is responsive when growing i.p. whether rIL-2 is administered at 550 IU/dose once or on a daily × 5 schedule for 4 weeks. All animals bearing i.p. Renca cells show approximately 180% test/control extension of lifetime. This is a significant result and may be due to the time afforded the animals to mount an appropriate antitumor immune response in the peritoneum and to the degree of immunogenicity of this tumor (Salup et al., 1985). However, this result, as with extension of lifetime data used, perhaps, too optimistically during preclinical evaluation of chemotherapeutics, does not translate to "cures" and should be maintained in perspective.

In addition to oncological parameters of tumor growth rates and immunogenicity, the second important factor which complicates evaluating i.p. tumor models in relation to immunotherapy is the uniqueness of the peritoneal immune response. It is possible, for example, to imagine the difficulty of mounting an inflammatory type of reaction against tumor cells growing in semisolid or fluid ascites, and peritoneal access to T cells is probably restricted. In fact, supplementing the immune cell population of the peritoneal cavity using antigen-specific, adoptive immunotherapy with CTLs (syngeneic splenocytes incubated ex vivo with irradiated P815 cells and rIL-2) appears to extend lifetime about 150% only if the rIL-2-stimulated CTLs are administered i.p. on the same day as i.p. tumor cell inoculations (Mills et al., 1980). Similar effects are even observed in nude mice with nonspecific (LAK) cell adoptive immunotherapy of i.p. human ovarian carcinoma xenografts (Ortaldo et al., 1986).

b. "METASTATIC" TUMOR MODELS. Metastatic tumor model rigor, defined operationally in relation to responsiveness to therapy, varies in experimental rodent tumors with cancer type and with protocols of the particular model. For example, in the very aggressive, spontaneously metastatic pancreatic tumor of the C57B1 mouse, PAN-02 (Corbett et al., 1984), rIL-2 has very little effect when administered in MTD bolus doses for weeks after initial s.c. tumor cell implantation. This tumor is also unresponsive to most single-agent chemotherapeutics and can thus be classified as very "rigorous."

Models in which the tumor cells metastasize from primary implants at a very low rate can be made more rigorous by artificially introducing tumor cells into the peripheral circulation for pulmonary trapping (Fidler, 1973). Such experimental metastases models are less "rigorous" than aggressive, spontaneously metastatic tumors, but they are more amenable to experimental manipulations and, by experimentally implanting many tumor cells in the lungs, protocols can be designed that result in host mortality within about 4 weeks. Pulmonary tumor load defines model rigor in this case, and, because high-dose rIL-2 induces immune cell activation/recruitment in the lung, it is not unexpected that the experimental metastasis B16 melanoma model is responsive to moderate-/high-dose rIL-2 therapy (Rosenberg et al., 1985a).

An example of the "least rigorous" metastasis model is the primary tumor (hindlimb) amputation protocol which allows spontaneously metastasizing B16–6 cells to then kill the host 10 to 12 weeks after primary implantation. While this model is more relevant to the human setting, it is one of the least rigorous rodent metastatic models because it allows for long-term exposure to tumor antigens, stress induced by amputation, and low tumor burden when the therapy is initiated. With this protocol, rIL-2 gives marginal to significant responses with both moderate/high daily bolus doses and continuous infusion of rIL-2 (Talmadge et al., 1987).

D. Combining rIL-2 with Other Therapeutic Modalities

It has become clear that single-agent and single-modality therapy for the treatment of cancer will never be optimal. Thus, a natural progression of research in combination studies of rIL-2 with lymphoid cells, other lymphokines, cytokines, and cytotoxic chemicals has occurred during the past few years. In particular, the admixing of proteins promises to be an exciting and informative approach to immune therapy because nature has designed these molecules to operate via cascade networks and, presumably, in finite sequences. A synopsis of combination studies follows in historic sequence.

1. Combining rIL-2 with cells for adoptive immunotherapy. a. CTLS versus LAK + rIL-2. Initial approaches to adoptive immunotherapy of cancer in animal models almost all used the strategy of infusing specifically sensitized (immune) syngeneic lymphocytes [or T cell clones (Matis et al., 1986)] to mediate regression of well-defined, established, transplantable tumors (Borberg et al., 1972; Rosenberg and Terry, 1977). When rIL-2 became available in quantities sufficient for therapeutic use, studies with these immune cells in conjunction with rIL-2 in vivo rapidly followed (Cheever et al., 1984a,b; Donohue et al., 1984b; Palladino et al., 1984), and in all cases, antitumor efficacy was improved with the addition of rIL-2 to treatment regimens. In fact, Ettinghausen and Rosenberg (1986) verified the basic concepts that sustained presence of rIL-2 generated by i.p. injections thrice daily for periods of at least 6 days was optimal for proliferation of exogenously administered lymphoid cells.

There seems to be little argument that immune lymphocytes represent a much more effective population for adoptive immunotherapy in rodent tumor models than do nonimmune cells. However, because of the lack of

knowledge regarding the immunogenicity of human tumors, and because clinical protocols call for autochthonous peripheral blood lymphocytes cultured with rIL-2 in vitro (which could be immune or nonimmune in nature), preclinical investigations largely from one group at the National Institutes of Health have been focused on the nonimmune cell approach using splenocytes from naive animals to generate LAK cells in vitro. Rosenberg's group is concerned with helping develop pharmacological principles for this form of adoptive immunotherapy in concert with clinical studies.

Of the few other investigators who have compared immune CTLs with (nonimmune) LAK cells, Thompson et al. (1986) have conducted the most complete and representative studies. These investigators found that the culture of nonimmune, syngeneic murine spleen cells with rIL-2 (55 IU/ml for 2 to 3 days) generates LAK cells capable of lysing FBL-3 leukemia cells in vitro. The same LAK cells were able to mediate an antitumor effect in vivo in a peritoneal model of FBL-3 such that 10^7 LAK cells injected i.p. prevented lethal outgrowth of 10^2 FBL-3 leukemia cells from the peritoneum of about 50% of the mice. The same number of LAK cells were ineffective against 10^6 FBL-3 cells.

One convenient way to express and compare such results with other studies is to identify an "effector to target ratio," or the ratio of LAK cells to tumor cells, which in this case was 100,000:1. This type of data, coupled with the short lifetime and differing biodistribution patterns of exogenously administered LAK cells compared with CTLs (Ettinghausen et al., 1985) and the fact that immune spleen cells (CTLs) cultured under similar conditions and tested similarly give 100% cures in the FBL-3 model at effector to target ratios of 10:1, has led investigators to utilize LAK cells in combination with rIL-2 at effector to target ratios of 100 to 1000:1 in animal models.

Combination studies with LAK and rIL-2 in a weakly immunogenic, fast-growing and rapidly (11 day) lethal i.p. MCA-105 tumor model were performed in both the classic extension of lifetime manner and in an unorthodox fashion, namely, by sacrificing animals for visual scoring of i.p. tumor burden (Ottow et al., 1987). This latter analysis was performed because the investigators were interested in dose effects for rIL-2 in the adoptive immunotherapy mode, and the model is apparently too rigorous (i.e., too short a time-to-death) for classical evaluation. Results showed that tumor control was optimum in an rIL-2 and LAK dose-response fashion if the 5500-IU rIL-2 dose was split into four daily injections rather than given as a single daily bolus. They also found that the best effects were seen if all treatments were given i.p. Despite the short time-to-death of controls, rIL-2 alone gave greater than 150% (test to control) extension of lifetime, and the addition of rIL-2 LAK (+rIL-2) gave greater than 200% (test to control) results. Thus, as with single-agent rIL-2 therapy, fractionating the daily rIL-2 dose with LAK cells gives better efficacy than single daily bolus rIL-2 +LAK dosing. Also, the addition of LAK allows lower daily total rIL-2 dosing to achieve efficacy similar to high-dose rIL-2 alone.

When comparing the requirement for (apparently) continued presence of between 5 and 55 units rIL-2/ml for in vitro generation of LAK cells with what would be an average "continual presence" with fractionated in vivo doses, the rIL-2 concentrations found in the circulation during each of the 24-h periods in the high-dose rIL-2 study described above would be about equivalent. However, using the experimentally induced (i.v. injection) pulmonary micrometastasis variation of the MCA-105 sarcoma model, which is marginally sensitive to high-dose rIL-2 if treatment is initiated within 3 days of cell implant, Mule et al. (1985) showed that even 10-day established micrometastases were significantly reduced in number if rIL-2 was given at thrice-daily moderate doses (250 to 1000 IU) on the same day and for several days after 10^7 LAK cells were given. These doses of rIL-2 alone are ineffective in this model. Two doses of 10^7 LAK cells (separated by about 3 days) were more efficacious than one dose, even one large (near MTD) dose, of 10^8 LAK cells. Apparently, then, for rIL-2 + LAK therapy, at least in animal models, the rIL-2 doses can be cut back at least an order of magnitude (well below toxic levels) for therapeutic efficacy at or exceeding that obtained with rIL-2 alone.

Interestingly, in the studies described above, the investigators also showed that the LAK cell source did not have to be a syngeneic animal, i.e., they found that LAK cells from splenocytes of allogeneic (DBA = H2d) animals were as effective as splenocytes from syngeneic (C57B1 = H2b) animals. Shiloni et al. (1986) extended these observations and showed that, while allogeneic LAK cells do provide efficacy, they are measurably less effective than syngeneic LAK cells unless they are administered intraportally for treating hepatic metastases. Thus, cell-cell recognition does not appear to be restricted at major histocompatibility loci for LAK activity, in contrast to findings of H-2 restriction for specific (CTL) adoptive immunotherapy (Greenberg et al., 1981).

Ultimate success and clinical applicability for this form of adoptive cellular immunotherapy may depend on solving several basic problems: (*a*) the ability to reproducibly create/identify LAK cells or activity under quality controlled conditions, (*b*) the potential for additive effects of repeated courses of treatment, (*c*) the relative immunogenicity of human tumors, and (*d*) tumor histiotype and location.

The first problem is perhaps the hardest to deal with. That is, the in vitro sensitivity of different tumor types to LAK cytotoxicity does not appear to correlate with in vivo sensitivity, namely, while there were clearly more- and less-sensitive tumor cell types in culture, antitumor

responses to rIL-2 + LAK in vivo did not correlate with in vitro data (Papa et al., 1986b). Similar conclusions were gleaned by Gregg et al. (1987) using LAK and rIL-2 therapy in a guinea pig model of acute B cell leukemia, namely, that despite in vivo antitumor effects, lytic activity of LAK effector populations against the B cell leukemia targets could not be demonstrated. In this latter model, an added anomaly was found in that, while single-agent rIL-2 and single-agent LAK cells gave similar extensions of lifetime, admixing that LAK cell dose and rIL-2 doses in combination studies did not significantly extend lifetime, even in an additive fashion.

The second question, that of whether additive effects might be obtained with repeated courses of therapy, was approached in studies of rIL-2 + LAK cell therapy in i.p. models of the MCA-102 and 105 sarcomas. In these models, one cycle of immunotherapy consistently reduced tumor load but cured few if any mice with established tumors. Studies by Eggermont et al. (1987) showed that four consecutive cycles of treatment at weekly intervals did not increase survival when compared to mice treated with only one cycle of treatment (once again using the MCA-102 and 105 i.p. model described above). In a follow-up study, Eggermont and Sugarbaker (1987) investigated potential reasons for this by repeatedly challenging mice with rIL-2 + LAK cell therapy (six "immunizations") prior to i.p. tumor cell injections. After implanting tumor, rIL-2 + LAK cell therapy was totally ineffective even when evaluated using the visual scoring technique. These investigators found that the serum for LAK + rIL-2 animals contained antibodies to LAK cell surface antigens (which could lyse the LAK cells) and "serum inhibitors" of rIL-2 which abrogated antitumor efficacy.

Because they were concerned with the relevance of rodent rIL-2 + LAK adoptive immunotherapy vis-a-vis immunogenicity and human tumors, Papa et al. (1986b) further adapted their artificial pulmonary metastasis model to what they defined as "weakly" and "nonimmunogenic" tumors. Criteria for a particular tumor model being immunogenic were (*a*) the ability to immunize syngeneic mice such that, after hindlimb (primary tumor site) amputation, reimplanted tumors would be rejected and/or (*b*) animals could be "vaccinated" with *C. parvum*/viable tumor cell mixtures at one site and a subsequent challenge resulted in no tumor progression at a separate site. While these criteria could inaccurately show that tumors that could induce immune suppression (e.g., by blocking antigen shedding, etc.) are nonimmunogenic, they do, nonetheless, provide rationale for grading tumor immunogenicity. And, within the limitations of the definition, all tumor types responded similarly to rIL-2 + LAK therapy (Lafreniere and Rosenberg, 1985a; Papa et al., 1986b).

The studies mentioned above also afforded an opportunity to determine whether this form of therapy worked equally well with tumors of varying histological type. This was the case for sarcoma, adenocarcinoma, and, as previously shown, melanoma (Mule et al., 1986a,b). Similarly, the successful application of rIL-2 + LAK to tumor metastases does not appear to depend upon tumor location. That is, hepatic metastases are as amenable to treatment as are pulmonary metastases (Lafreniere and Rosenberg, 1985b). However, in view of the organ-homing properties of LAK cells, one would not necessarily expect lung or liver metastases to be refractory. Brain metastases might provide a quite different result.

In summary, the currently available, preclinical efficacy data that directly compare rIL-2 therapy with rIL-2 + LAK cell adoptive immunotherapy come primarily from one group of investigators whose goals were to help establish parameters for the use of adoptive cellular immunotherapy in the clinic. A majority of the data are generated with a moderately immunogenic, chemically transformed murine sarcoma cell, MCA-105, which consistently responds in a dose-related manner to the combination of rIL-2 and LAK cells. This tumor (and several other murine tumors evaluated) does not respond to LAK cells alone but does respond to high-dose rIL-2 alone if the tumor has been implanted for 10 days prior to therapy (enough time for the host to mount an immune response). It is impressive that rIL-2 + LAK cell therapy is effective at reducing the numbers of metastatic nodules in both pulmonary and hepatic metastasis models, and this phenomenon does appear to correlate with extension of lifetime, but the facts that exogenously administered LAK cells tend to "home" to lung and liver (Ettinghausen et al., 1985) and that repeated cycles of therapy generate anti-LAK and anti-rIL-2 activities in the syngeneic host (Eggermont and Sugarbaker, 1987) must be kept in mind to decrease the risk of overinterpreting the data in clinical correlations.

Two general conclusions can be made from the cumulative data. First, high-dose rIL-2 alone is as effective as moderate-dose rIL-2 + LAK cell therapy. Second, as long as one is working with transplantable rodent tumors, the success of therapy with nonimmune LAK cells and rIL-2, while not as influenced by tumor cell and donor (lymphoid) cell factors as other forms of adoptive immunotherapy, is probably most limited by the ability to reproducibly generate LAK cells in vitro and to then deliver those LAK cells to the tumor in the presence of rIL-2.

b. rIL-2 WITH ALLOGENEIC CELLS (ALLOADJUVANT IMMUNOTHERAPY). Steller et al. (1986) showed a marked augmentation of the immunotherapeutic effects of moderate-dose rIL-2 (2,800 IU, twice daily × 6 from day 0) when they promoted a tumor-localized, but syngeneic, tumor-nonspecific immune response by admixing syngeneic and allogeneic tumor cells prior to inoculation into the animal. While basically a modification of the bacillus Calmette-Guerin vaccine (BCG) peritumoral ad-

juvant immunotherapy approach, the logic behind this protocol, confirmed by Sherburne et al. (1987) using simultaneous L1210 and EL4 tumor cell inoculations in DBA/2 mice (syngeneic for L1210) and rIL-2 therapy at 2800 to 5600 IU, every 2 days × 5 or daily × 10, appears valid. In fact, increased, nonspecific, tumor-localized inflammatory response mechanisms should be kept in mind when evaluating studies in which synergies are seen between xenobiotics and rIL-2.

2. rIL-2 with other lymphokines and cytokines. a. rIL-2 + IFNs. Antitumor effects of IFNs have been reported in animal models (Gresser and Bourali, 1970; Kataoka et al., 1985) as well as human studies (Spiegel, 1987). The mechanism(s) of this activity is unknown, but the IFNs can directly inhibit tumor cell growth (Brunda and Wright, 1986), promote reversal of the malignant phenotype (Hicks et al., 1981), and enhance expression of Ia or HLA-DR antigens (Steeg et al., 1982; Basham and Merigan, 1983; Wan et al., 1987), β-2-microglobulin (Heron et al., 1978), and Fc receptors (Aguet et al., 1981) on normal as well as tumor cell surfaces (Weber et al., 1988). IFNs can inhibit T cell suppressor activities (Knop et al., 1987), modulate B cell function (Goodman, 1987), and activate macrophages (Chen and Najor, 1987). Because IFN-γ has been detected in the circulation of patients after rIL-2 therapy (Lotze et al., 1985), and because IFN-γ induces IL-2 receptor expression on peripheral blood monocytes (Holter et al., 1986), studies of rIL-2's antitumor synergies with IFN-γ were called for.

The problem from an experimental standpoint relates to the high degree of species specificity of IFNs. Production of large amounts of murine IFNs for preclinical studies has lagged behind production of recombinant human IFNs. However, when investigators have worked with murine IFNs, they find that the combination of rIL-2 with either murine IFN-α or IFN-β exhibits much increased efficacy compared to either agent alone (Iigo et al., 1988). The synergy observed did not occur in immune-compromised animals. What is interesting about these types of studies is that there may be model-related clues as to the potential mechanisms of synergy because, in the study mentioned above, combination rIL-2 and recombinant murine IFN-γ did not show synergy, whereas using a melanoma model, Silagi et al. (1988) showed a potent synergy between rIL-2 and mouse IFN-γ. Because there are known to be two different IFN receptors [one for α/β and one for γ (Branca and Baglioni, 1981)], it is possible that the receptor or its differential expression on different immune cells is crucial to therapeutic synergy seen as a function of different tumor types.

In vivo generation of NK cell activity is an autonomous function of the bone marrow (Haller et al., 1977). In vitro studies show that rIL-2 and IFNs are able to influence the growth and differentiation of precursor cells to cytolytic NK cells and, using a bone marrow transplantation model in B6D2Fl L1 mice, which are congenitally low in NK cell precursors, Riccardi et al. (1986) were able to demonstrate synergy between IFN-treated bone marrow and rIL-2-treated L1 recipient mice when they observed earlier and higher NK cell re-constitution, provided treatments were performed during the first 3 days after bone marrow transplantation. Similar studies by Shalaby et al. (1985) showed that the combined therapeutic regimen of IFN-γ and rIL-2 augments NK activity in mice, and recently Eggermont et al. (1988) showed that the IFN-inducer, 2-amino-5-bromo-6-phenyl 4-pyrimidinone, shows synergy with rIL-2 in generating LAK cell activities in peritoneal exudates. 2-Amino-5-bromo-6-phenyl 4-pyrimidinone also showed synergy with rIL-2 and LAK cells in early implant murine tumor models if the inducer was administered before the adoptive immunotherapy.

b. rIL-2 + TNF. Because rIL-2 induces TNF-α production in human peripheral blood monocytes (Nedwin et al., 1985), TNF up-regulates the expression of normal cell surface as well as tumor cell surface histocompatibility antigens (Collins et al., 1978; Pfizenmaier et al., 1987), rIL-2 augments the cytotoxicity of monocytes (Malkovsky et al., 1987), and TNF/cachetin plays a central role in acute inflammatory states such as septic shock (Cerami and Beutler, 1988), studies of the combination of rIL-2 and TNF in s.c. murine tumor models were also logical. In particular, findings that certain murine tumors (such as the B16 melanoma) cause a transient stimulation followed by lasting suppression of NK activity about 1 week after tumor implantation (Lala et al., 1985) suggested that appropriate immune cell activation and perhaps the sequence and timing of combination studies are keys to successful lymphokine therapy (Winkelhake et al., 1987). Such studies with human rIL-2 and human recombinant (r)TNF in mice show a striking synergy in that the simultaneous administration of MTD daily bolus doses of TNF and even 90-fold less than MTD doses of rIL-2 completely blocked the growth of B16 melanoma, L1210 leukemia, P815 mastocytoma, and EL-4 lymphoma cells (Winkelhake et al., 1987). Synergy was apparently dependent upon tumor burden and relative degree of immunogenicity because complete inhibition of tumor take correlated with the day of initiation of combination therapy, i.e., blockage of tumor growth was seen for B16 melanoma only if therapy was initiated within 1 day of implanting 10^6 cells, while for L1210 leukemia and P815 mastocytoma, the day of therapy initiation could be delayed until 3 to 5 days after implanting 10^6 cells. The admixed combination of rIL-2 and TNF was also effective in the B16 melanoma experimental pulmonary metastases model but not effective for B16 cells implanted i.p., even with i.p. therapy. Similar results have been recently reported by McIntosh et al., 1988).

These observations have been extended to evaluate potential sequence/timing effects, and it was discovered that, for rodent s.c. tumor models, the sequence of TNF (daily × 3) followed by rIL-2 (daily × 11) was able to block and even cure B16 melanoma tumors that had been implanted for 5 or 10 days prior to initiating therapy (Zimmerman et al., 1989b). Nishimura et al. (1987) reported similar synergistic activity by intratumoral sequential treatment with TNF and rIL-2. Of particular interest is that this potent synergy is apparently not observed when using rIL-2 with murine rTNF using the same tumor models (H. Brunda, et al., manuscript submitted for publication). One possible explanation lies in species specificity such that, unlike human rTNF in the mouse, murine rTNF activates murine T cells (perhaps causing a hypoactivation and actual inhibition of rIL-2 efficacy). Human rTNF does activate human T cells (Scheurich et al., 1987), and clinical studies of rIL-2 and rTNF have not shown as dramatic results as those found with mice.

While mechanisms underlying the potent synergy of these two human proteins in mice are very poorly understood, differing results as a function of species are extremely provocative in that they suggest both that immune network cascades and sequences of events can be manipulated with therapeutic benefit and that there are obvious strategies for designing new cytokines with cellular activities in man similar to those seen with human proteins in the mouse.

c. rIL-2 + COLONY-STIMULATING FACTORS. As of this writing, studies combining rIL-2 with colony-stimulating factors are in their infancy. The colony-stimulating factors appear to be able to stimulate bone marrow cells much more specifically than rIL-2, but more data are needed. In any event, the potential for selectively stimulating the production of subpopulations of leukocytes as a mechanism for immune system augmentation is overwhelming and is the key hope for biological therapeutics.

3. rIL-2 with chemotherapies. In any combination therapy, there is generally a rationale for schedule and sequence dependency. So too there appear to be immunopharmacological rationale to explain findings of schedule and sequence dependence of rIL-2 in combinations with chemotherapies.

a. rIL-2 + IMMUNOSUPPRESSIVE DRUGS. The important component of combination therapies with rIL-2 and most chemotherapy regimens is that chemotherapy is many times (but not always) immunosuppressive. Thus, alkylating agents such as cytoxan (CTX) and the nitrosoureas (nucleic acid analogs such as 6-mercaptopurine and azathioprine, 5-fluorouracil and cytosine arabinoside, folate agonists such as methotrexate, *Vinca* alkaloids such as vincristine, and all corticosteroids) are generally immunosuppressive (reviewed by Kempf and Mitchell 1984, 1985).

There are several theoretical reasons for using immunosuppressive drugs in combination with rIL-2, but the most logical and experimentally verifiable one is based on the fact that most chemotherapeutics selectively suppress immune system functions, thereafter allowing the experimentalist to augment immunity with rIL-2. Agents that are known to selectively suppress immunity include CTX at low doses, colchicine, adriamycin (duanorubicin), and some prostaglandin antagonists at specific doses/schedules. The basic mechanism appears to be indirect in that they preferentially inhibit Ts cells or precursors of CD4-type cells, but CTX is thought to decrease tumor antigen-specific Ts cells as well (Nomi et al., 1984).

Conversely, cisplatin can increase immunity apparently by increasing the potency of nonspecifically cytotoxic macrophages (Kleinerman, 1980). Another example of this type of up-regulating mechanism is seen in the basic observation whereby cures of mice bearing the MOPC-315 tumor can be obtained using low-dose CTX at stages of tumor growth when antitumor immunity is depressed (Mokyr et al., 1979; Mokyr and Dray, 1983). Theoretically, this occurs because the CTX reverses macrophage-induced suppression and allows the appearance of immunopotentiating T cells (Ye and Mokyr, 1984; Ye et al., 1984).

Whatever the basic mechanism, there is, in fact, a definable synergy between CTX and rIL-2 (Silagi and Schaefer, 1986; Papa et al., 1988). And, while schedule/sequence phenomena have not been extensively studied, synergies between rIL-2 and CTX are found in animal models when the lymphokine is given at approximately the same time or after chemical therapeutic (personal observations). In this case, one of the mechanisms may be related to our finding that CTX delays the rodent's anti-rIL-2 antibody response such that several rounds of sequential combination rIL-2 + CTX treatments give additive effects.

There is, of course, a less complex argument for administering chemotherapies before rIL-2 and that is as a tumor-debulking technique. Chemotherapy can reduce the sheer number of viable tumor cells that the immune system has to contend with, and subsequent administration of rIL-2 would initiate a "potato peel" effect wherein drug destroys s.c. neoplastic cells on the tumors' vascularized periphery resulting in lymphocytic infiltrates. Evans (1983) described such a tumor-debulking effect for CTX. With less tumor bulk, rIL-2 could then activate tumor-infiltrating lymphocytes to augment a localized inflammatory response (tumor cell surface antigen-specific or nonspecific) which could subsequently facilitate the second-cycle drug access to cells closer to the tumor's necrotic region(s).

This is a particularly tantalizing hypothesis which seems to be supported by data suggesting greater effectiveness of CTX in more immunogenic tumors, although

even this tendency is controversial (Evans, 1978). Merely slowing tumor growth would also afford more time for the immune response to act against immunogenic tumors, and this hypothesis suggests that care must be taken in interpreting efficacy results when animals/patients have been pretreated with chemotherapies. A lot depends upon immune status when rIL-2 is administered.

b. rIL-2 + NONIMMUNOSUPPRESSIVE DRUGS. With very immunogenic tumors in animals, the most effective chemotherapeutic agents are those that are least immunosuppressive. Agents that are also antibiotics such as bleomycin, mithramycin, doxorubicin and its congener, daunorubicin, and some alkylating agents such as dacarbazine and bisulfan have little immunosuppressive activity (reviewed by Mitchell, 1988). Only in these cases or in the case of fluorinated quinilones which induce hyperproduction of rIL-2 (Reisbeck et al., 1989) is there a teleological reason to concomitantly administer chemotherapy with rIL-2.

c. rIL-2 + IMMUNE SYSTEM-AUGMENTING DRUGS. Several chemotherapeutics such as adriamycin, dacarbazine, nitrosoureas, and methotrexate can apparently alter tumor cell membranes and make them more immunogenic, perhaps by acting as a hapten or by selecting more immunogenic, drug-resistant mutants. In fact, a possible general mechanism of action in the case of all combinations with cytotoxic chemicals is that subsequent administration of rIL-2 may help promote a localized, nonspecific immune "clean-up" of damaged tumor cells. In these cases, logic dictates combination therapy or even administering rIL-2 after chemotherapy, but there is not much reason to believe that chemotherapy should follow rIL-2.

d. rIL-2 AS AN ANTITOXIC IN CHEMOTHERAPY. A beneficial effect of rIL-2 in reversing or blocking the toxicities of chemotherapeutics has been suggested by the work of Talmadge (1985, 1987; Talmadge and Herberman, 1986). The stimulation of bone marrow colony-forming units in mice appears to protect them from lethal myelosuppression induced by chemo- or radiation therapy. The situation is complex, however, in that, while rIL-2 and other cytokine therapy was found to induce colonies and correlate with potentiation of chemotherapy, T cells were involved because this effect was not seen in nude mice. More studies are needed in this area because the role of rIL-2 in mechanisms of nonspecific tolerance clearly need unraveling. If rIL-2 were able to induce a nonspecific tolerance to toxic xenobiotics, then administration of rIL-2 prior to the xenobiotic would make sense, especially if chemotherapeutic toxicity was a major limiting factor to efficacious treatment. This logic could be equally applied when attempting to schedule rIL-2 in a combination regimen with radiotherapy for cancer. In this case, the optimal rIL-2 schedule would be not only prior to but also after radiotherapy.

e. rIL-2 + ANTI-INFLAMMATORY DRUGS (INDOMETHACIN). In a provocative series of studies based on the purported role of prostaglandins in immunosuppression in cancer (Murray et al., 1983; Young et al., 1984; Lala et al., 1985), Lala and Parhar (1988) showed that the combination of chronic indomethecin and repeated rounds of rIL-2 therapy activates killer cells of the broadest spectrum (compared with rIL-2 alone) and geographic distribution. This effect included the tumor site and resulted in lasting cures of experimental B16 melanoma metastasis. NK cells play a key role in the mouse models and likely contribute to toxicity by release of prostaglandins as well. However, these approaches are promising because they further suggest that efficacy and toxicity may be separated, at least in time. And, in fact, there is no reason not to speculate that efficacy, which is based on an immune response with memory, can be separated in time from immediate, nonspecific, and nonanamnestic toxic effects.

f. rIL-2 AS A VACCINE ADJUVANT. Using the National Institutes of Health test for rabies vaccine potency, Nunberg et al. (1989) found that daily systemic administration of rIL-2 to mice in conjunction with inactivated rabies virus increased vaccine potency at least 25-fold. Enhanced protection as measured by survival following challenge with virulent rabies virus did not correlate with increased virus-neutralizing antibody titers, however. Similar findings were obtained in a study in a guinea pig model using herpes simplex virus vaccine in which the authors (Weinberg et al., 1986) conclude that, while not correlating with elevated antibody titers, enhanced vaccine potency did correlate with rIL-2 enhanced specific cytotoxicity due to T cell immunity.

4. Triple combinations: rIL-2 + LAK and xenobiotics. As might be expected, there appears to be a substantial addition of effects between xenobiotics and adoptive immunotherapy with LAK + rIL-2. Thus, with the i.p. Renca model which results in extensive peritoneal carcinomatosis, hemorrhagic ascites, metastases to abdominal nodes, liver, spleen, and, in some animals, lungs, i.p. treatment with doxorubicin or LAK + rIL-2 results in 20 or 50% cures, while the combination of all three (drug, rIL-2, and LAK) results in a 90% cure rate (Salup and Wiltrout, 1986a,b). Additional studies are solely needed in this important area which capitalizes on different mechanisms of synergy between any two of the three therapeutic components.

IV. Efficacy: rIL-2 Therapy for Infectious Diseases

A. rIL-2 for Bacterial Infections

Prophylactic administration of a single high dose (about 5500 IU) protects mice from an otherwise lethal, acute *E. coli* infection (Chong, 1987). However, this phenomenon is route specific, that is, efficacy is seen only when rIL-2 and bacteria are administered by the same route. This perplexity exists as a caveat for virtually

all infectious disease models and may be related to the fact that host resistance to infections is dramatically enhanced by even ng doses of endotoxin, teichoic acids, fungal surface carbohydrates, etc. (e.g., Chong and Huston, 1987). Perhaps best studied with endotoxin, the nonspecific enhancement, known as "pharmacological tolerance," is time dependent and requires about 18 h to develop fully. Thus, in infectious disease models in general, route and schedule of therapy and microbial challenge are keys to efficacy and extreme care must be taken to assure that animals do not receive contaminants (e.g., endotoxins) which potentially are found at low concentration on laboratory equipment and in reagents.

One other controversial theme that comes from the more recent studies is that the therapeutic efficacy of rIL-2 in infectious disease models, especially for extracellular microbes such a *Klebsiella pneumoniae,* may not be due to NK cell activation or the presence of antigen (Iizawa et al., 1988). Rather, rIL-2 therapy for certain diseases may be advantageous simply because it substitutes for disease-associated inhibition of autologous IL-2.

B. rIL-2 Effects in Microbial Immunosuppression Models

High doses of *Mycobacterium bovis* BCG, injected i.v. in mice, rats, and guinea pigs, cause a T cell unresponsiveness (Collins et al., 1987; Lamoureux and Poisson, 1974). Both the specific delayed-type hypersensitivity skin reactions and T cell responsiveness to lectins reverse when low doses (hundreds of units) of rIL-2 are administered to *M. bovis* BCG-infected mice (Colizzi, 1984). What is unique about this finding is that the rIL-2 treatments resulted in a monophasic dose response if treatments were carried out daily for greater than 4 days and before 8 to 12 days postinfection, i.e., during a period in which Ts cells are probably not operative (Collins and Watson, 1979). These authors propose that the disease-associated defect is due not to the presence of Ts cells per se [as is proposed to be the major BCG-induced suppressive mechanism in both humans and animals (Nakamura and Tokunaga, 1980; Turcotte, 1981)] but rather to the action of a substance that suppresses rIL-2 production, as has been shown to be the T cell lesion in *Mycobacterium lepraemurium*-induced immune suppression (Hoffenbach et al., 1983). Circumstantial support for this was recently presented by Jeevan and Asherson (1988) when they confirmed that rIL-2 efficacy (limiting the replication of *M. lepraemurium* in vivo) is not related to increased NK cell activities, the presence of antigen, or an increased number of phagocytic cells. In fact, reduced autologous IL-2 production is seen in unresponsiveness to several contact-sensitizing agents such as oxazolone and picryl chloride (Asherson et al., 1983).

Similarly, mice infected with *Trypanosoma cruzi* (experimental Chagas disease) exhibit an early and profound suppression of parasite-specific and nonspecific immune responses (Clinton et al., 1975; Tarleton and Kuhn, 1984). One effect of this experimental disease is a decrease in IL-2 production (Harlen-Bellan et al., 1983), and rIL-2 administration reverses both specific and nonspecific immune unresponsiveness if administered during the period of marked immune suppression, between 7 and 16 days postinfection (Chromomanski and Kuhn, 1985, 1987).

In the case of toxoplasmosis, in which immunosuppressive effects of the intracellular parasite *Toxoplasma gondii* are less clear (i.e., NK cell activity is actually enhanced during the infection process), Remington's group showed that rIL-2 doses as low as 5 IU administered every 2 days to mice during the first week after a lethal challenge with *T. gondii* significantly decreased mortality and tissue cyst formation (Sharma et al., 1985). Despite this significant effect of low-dose rIL-2, these investigators were unable to identify a cell-based phenomenon such as significantly increased NK activity, increased macrophage cytolytic activity, or reversal of *T. gondii*-induced, concanavalin A nonresponsive lymphocytes, and these results remain provocative from both the mechanistic and pharmacological standpoints.

Clearly many more studies are needed to even begin to understand whether anti-infectious disease properties of rIL-2 are at all based on mechanisms similar to those operating in antitumor efficacy and whether increased sensitivity of animals to the toxic effects of rIL-2 when they have an infection (K. Chong, personal communication) are tied in some way to efficacy in infectious diseases.

C. Adoptive Immunotherapy for Infectious Disease

In an adoptive transfer model of murine cytomegalovirus, Reddehase et al. (1987) obtained immune lymphocytes from animals recovered from a cytomegalovirus infection and injected them into γ-irradiated (immune-depleted) animals with chronic viral infections. In these latter animals, cytomegalovirus replicates in, and can be plaque-assayed from, the lungs, and rIL-2 was shown to enhance the antiviral function (i.e., a reduced lung infection) of defined, but insufficient numbers of, transferred lymphocytes. The antiviral effects were not seen in the adrenal glands of these same animals.

Similarly, in an experimental model of herpes virus infection with antigen-specific adoptive immunotherapy, Rouse et al. (1985) showed that splenocytes incubated with ultraviolet light-inactivated herpes simplex virus type 1 for 5 days in vitro were much more effective than nonimmune splenocytes when administered to animals 2 h after infection with the virus. The addition of rIL-2 1 h prior to infection and in gelatin 6, 24, and 48 h after infection augmented the immune cell's ability to reduce viral plaques. No protection against systemic challenge with herpes simplex virus type 1 was seen with rIL-2 alone in this model. However, Weinberg et al. (1986) did

show lower numbers of lesions and prolonged survival in a guinea pig model of acute genital infection with herpes simplex virus type 2.

The latter studies also showed that the animals that were protected against herpes simplex virus type 2 did not seroconvert, i.e., they did not acquire immunological memory, antibody, or sensitized T lymphocytes. The frequency of spontaneous recurrence was, however, not affected in this model, and these investigators found a biphasic dose-response curve in which protection was lost at high doses of rII-2, perhaps due to inhibitory effects on IFN-γ production (Pearlstein et al., 1983).

V. Conclusions

Biological response modifiers represent a new class of agents for cancer therapy and promise to become a fourth treatment modality in addition to surgery, radiotherapy, and conventional chemotherapy. During the short, 5-year history of preclinical testing of rIL-2, a remarkable amount of literature has accumulated in concert with deservedly optimistic excitement over the new drug's potential as a biotherapeutic. As was the case for the IFNs, this literature is made possible by a healthy, free flow of material and information among industrial and governmental and academic scientists, and several conclusions can already be made because of this relatively new approach to drug development.

First, there is no question that rIL-2 leads the way as a novel biological drug in immunotherapeutic approaches to cancer and infectious diseases. The central role of rIL-2 at physiological concentrations in promoting T cell growth and in activating and recruiting other lymphoid elements in immune responses is, we believe, now well established.

Second, it should come as no surprise that rIL-2, when used at pharmacological doses, elicits toxic responses. As with classical anticancer drugs, it appears that at least some of the mechanisms that contribute to toxicity may also play key roles in antitumor efficacy as well. For example, VLS may translate to tumor vascular bed effects and may actually contribute to tumor necrosis and regression for certain types of tumors. This toxicity-efficacy relationship may not be surprising to oncologists used to studying chemotherapeutics, but it may come as some surprise that there is ample reason to believe that toxicity and efficacy may be separated for rIL-2 by learning more about how this paracrine hormone plays its part in immune regulation. Thus, future studies of the mechanisms behind wanted and unwanted effects of (now nine) interleukins are sorely needed to provide new concepts for cancer therapy in fashions analogous to those discovered with conventional xenobiotics.

In particular for rIL-2, it is easy to imagine that a major efficacy mechanism, namely, a memory-based immune antitumor response, can be separated in time from nonspecific toxicity that occurs only during the period of treatment. Thus, much more work is needed even on the early time course of toxic manifestations, especially as they relate to fluid and lymphocyte flow. There are clues in the literature that something fundamental to the exhuberant pharmacological activities of rIL-2 begins happening at approximately 4 to 6 days after initiating high-dose rIL-2 therapy (at least in animal models). This is quite different from the general scheme of events with systemic inflammatory responses and is likely an important clue to toxicity mechanisms. Meanwhile, for therapeutic utility, antitumor effects may occur weeks or even months after rIL-2 treatment is terminated.

Third, the rapid blood clearance of rIL-2 has probably contributed to some delay in understanding how best to use this lymphokine as a therapeutic, but important clues and information are now being obtained with second generation rIL-2 (e.g., polyethylene glycol-modified rIL-2) and in continuous infusion studies. Early efficacy data showed that, while some animal models are sensitive enough to respond to low doses, administration of more rIL-2 for longer periods generally gave more efficacious results. When multiphasic dose responses were evaluated in vivo, they were usually triphasic, and optimal efficacy was seen at low- and high-dose levels. This, along with slow-release and continuous infusion data, suggests that efficacy results are due to multiple, interacting processes and are largely affected by total exposure period (area under the blood clearance curve) as well as the shape of the clearance curve with time. Thus, because the host is the "drug," there appear to be threshold levels for toxicity and efficacy that are intricately tied to concentration-over-time rather than simple dose-response parameters which drive many conventional drug pharmacodynamics. These data also suggest that it may be possible to minimize toxic (unwanted) effects that are clearly related to dose intensity and duration by developing analogs of rIL-2 with uniquely different pharmacokinetic profiles.

Immunotherapy has its forte in situations in which an insufficient or inappropriate immune mechanism is already at work. In virtually all animal models of neoplasic or infectious disease, this is the case. While this is not so clearly the case for human cancer (and this makes the animal model efficacy studies biased in favor of rIL-2), there are still major clues within the preclinical data that should be useful in testing rIL-2 (and other lymphokines) in the clinical setting. For example, weakly immunogenic models and therapies involving immune-suppressed animals suggest that expansion of the host's lymphocytes (whether antigen-specific CTLs or less specific LAK cells) can have a beneficial antitumor effect. These findings coupled with the leukopoietic activities of rIL-2 and recent findings of a strong sequence dependence between other cytokines and rIL-2 all suggest that it may be possible to manipulate the overall immune "steady state" of the host. In fact, it may be possible to capitalize on mechanisms of nonspecific, pharmacological tolerance simply through the proper pharmacological use of cyto-

kines. Preclinical studies with rIL-2 in the infectious disease realm strongly support this conclusion and suggest that rIL-2 may have a very important role in therapy (and prophylaxis) for infections in patients receiving immunosuppressive chemotherapies or radiation therapy.

A chief difference between animal tumor models and human cancer is in the time course of the disease. For most animal tumors, the time window during which it is possible to augment the immune response without adding exogenously expanded cells is relatively short. Even with LAK cell additions, animal tumors grow so rapidly that it is usually only a matter of weeks before they can overwhelm immune responses. Also, there is apparently a limit beyond which stimulating lymphoid cell production becomes counterproductive, if not toxic. Thus, with animal models one is forced to give high and continuous doses of rIL-2 to see optimal efficacy. This may or may not be viewed as suggesting that animal tumors are more rigorous than their slow-growing human tumor counterparts. But it seems that to really evaluate rIL-2's relevance for human use, one needs a model with the exact parameters that most animal models are selected against, i.e., a spontaneous, slow-growing and metastatic tumor.

Of course there is no animal disease model that is ideal, but there may well be an ideal combination of rIL-2 with other lymphokines, cytokines, and chemotherapeutic drugs. Clearly, the development of a pure rIL-2 has allowed us to begin to examine the pharmacological principles of immunotherapy to an extent never possible in previous approaches such as with BCG therapy. With the advent of these types of pure biological response modifiers comes the increasing hope that the experimentalist will be able to meaningfully orchestrate immune responses with proteinaceous cytokines in the very near future.

Acknowledgments. We are very grateful for thoughtful comments and discussions with Drs. J. Young and L. Houston. While attempting to be comprehensive, we have occasionally limited this review. Thus, we may have unintentionally overlooked work by investigators in the field and apologize for such oversight.

REFERENCES

ABUCHOWSKI, A., KAZO, G. M., VERHOEST, C. R., VAN ES, T., KAFKEWITZ, D. NUCCI, M. L., VIAU, A. T., AND DAVIS, F. F.: Cancer therapy with chemically modified enzymes. I. Antitumor properties of polyethylene glycol-asparaginase conjugates. Cancer Biochem. Biophys. **7:** 175–186, 1984.

AGUET, M., VIGNAUX, F., FRIDMAN, W. H., AND GRESSER, I.: Enhancement of Fc receptor expression in INF-treated mice. Eur. J. Immunol. **11:** 926–930, 1981.

ALEXANDER, J. T., SARIS, S. C., AND OLDFIELD, E. H.: The effect of IL-2 on the blood-brain barrier in the 9L gliosarcoma rat model. J. Neurosurg. **70:** 92–96, 1989.

ALEXANDER, P. E., DELORME, E. J., AND HALL, J. G.: The effect of lymphoid cells from the lymph of specifically immunised sheep on the growth of primary sarcomata in rats. Lancet **1:** 1186–1189, 1966.

ANDERSON, T. D., AND HAYES, T. J.: Toxicity of human recombinant interleukin-2 in rats. Lab. Invest. **60:** 331–346, 1989.

ANDRIOLE, G. L., MULE, J. J., HANSEN, C. T., MARSTON LINEHAN, W., AND ROSENBERG, S. A.: Evidence that lymphokine-activated killer cells and natural killer cells are distinct based on a analysis of congenitally immunodeficient mice. J. Immunol. **135:** 2911–2913, 1985.

ARONSON, F. R., LIBBY, P., BRANDON, E. P., JANICKA, M. W., AND MIER, J.: IL-2 rapidly induces natural killer cell adhesion to human endothelial cells. J. Immunol. **141:** 158–163, 1988.

ASANO, Y., AND TADA, T.: Generation of T cell repetoire: two distinct mechanisms for generation of T supressor cells, T helper cells, and T augmenting cells. J. Immunol. **142:** 365–373, 1989.

ASHERSON, G. L., ZEMBALA, M., COLIZZI, V., AND MALKOVSKY, M.: Lymphokines and antigen-specific T-cell products in contact sensitivity. Lymphokines **8:** 143–173, 1983.

ATHENS, J. W., RAAB, S. O., HAAB, O. P., MAUER, A. M., ASHENBRUCKER, H., CARTWRIGHT, G. E., AND WINTROBE, M. M.: Leukokinetic studies. III. The distribution of granuloyctes in the blood of normal subjects. J. Clin. Invest. **40:** 159–164, 1961.

AUKLAND, K., AND NICOLAYSEN, G.: Interstitial fluid volume: local regulatory mechanisms. Physiol. Rev. **61:** 556–698, 1970.

BACHIA, P., WILLIAMS, D. P., WATERS, C., WILLIAMS, J. M., MURPHY, J. R., AND STROM, T. B.: Interleukin 2 receptor-targeted cytotoxicity: interleukin 2 receptor-mediated action of a diphtheria toxin-related interleukin 2 fusion protein. J. Exp. Med. **167:** 612–622, 1988.

BARBUL, A., KNUD-HANSEN, J., WASSERBRUG, H. L., AND EFROM, G.: Interleukin-2 enhances wound healing in rats. J. Surg. Res. **40:** 315–319, 1986.

BASHAM, T. Y., AND MERIGAN, T. C.: Recombinant interferon-gamma increases HLA-DR synthesis and expression. J. Immunol. **130:** 1492–1494, 1983.

BEAUCHAMP, C. O., GONIAS, S. L., MENAPACE, D. P., AND PIZZO, S. V.: A new procedure for the synthesis of polyethylene glycol-protein adducts: effects on function, receptor recognition, and clearance of superoxide dismutase, lactoferrin, and alpha2-macroglobulin. Anal. Biochem. **131:** 25–33, 1983.

BERT, J. L., AND PEARCE, R. H.: The interstitium and microvascular exchange. *In* Handbook of Physiology—The Cardiovascular System, ed. by E. M. Renkin and C. C. Michel, vol. 4, pp. 521–547, American Physiological Society, Bethesda, MD, 1984.

BILLINGHAM, R. E., BRENT, L., AND MADAWAR, P. B.: Actively acquired tolerance of foreign cells. Nature (Lond.) **172:** 603–606, 1953.

BORBERG, H., OETTGEN, H. F., CHOUDRY, K., AND BEATTIE, E. J.: Inhibition of established transplants of chemically induced sarcomas in syngeneic mice by lymphocytes from immunized donors. Int. J. Cancer **10:** 539–547, 1972.

BRANCA, A. A., AND BAGLIONI, C.: Evidence that types I and II interferons have different receptors. Nature (Lond.) **294:** 768–770, 1981.

BRANDHUBER, B. J., BOONE, T., KENNEY, W. C., AND MCKAY, D. B.: Crystals and a low resolution structure of interleukin-2. J. Biol. Chem. **262:** 12306–12308, 1987.

BRUNDA, M. J., AND WRIGHT, R. B.:. Differential antiproliferative effects of combinations of recombinant IFNs-α and γ on two murine tumor cell lines. Int. J. Cancer **37:** 287–291, 1986.

BUBENIK, J., KIELER, J., AND INDROVA, M.: Local treatment with human recombinant rIL-2 inhibits growth of MC-induced sarcomas in syngeneic mice. Folia Biol. (Praha) **32:** 209–211, 1986.

BURDACH, S., SHATSKY, M., WAGENHORST, B., AND LEVITTI, L.: Receptor-specific modulation of myelopoiesis by recombinant DNA-derived IL-2. J. Immunol. **139:** 452–458, 1987.

BUTCHER, E. C., SCOLLARY, R. G., AND WEISSMAN, I. L.: Organ specificity of lymphocyte migration: mediation by highly selective lymphocyte interaction with organ-specific determinants on high endothelial venules. Eur. J. Immunol. **10:** 556–561, 1980.

BUTCHER, E. C.: The regulation of lymphocyte traffic. Curr. Top. Microbiol. Immunol. **128:** 85–122, 1986.

CANTRELL, D. A., AND SMITH, K. A.: The interleukin-2 T-cell system: a new cell growth model. Science (Wash. DC) **224:** 1312–1316, 1984.

CERAMI, A. C., AND BEUTLER, B.: The role of cachetic/TNF in endotoxic shock and cachexia. Immunol. Today **9:** 28–31, 1988.

CHEEVER, M. A., AND GREENBERG, P. D.: In vivo administration of interleukin-2. Contemp. Top. Mol. Immunol. **10:** 263–282, 1985a.

CHEEVER, M. A., GREENBERG, P. D., FEFER, A., AND GILLIS, S.: Augmentation of the anti-tumor therapeutic efficacy of long-term cultured T-lymphocytes by in vivo administration of purified interleukin 2. J. Exp. Med. **155:** 968–980, 1982.

CHEEVER, M. A., GREENBERG, P. D., IRLE, C. THOMPSON, J. A., URDAL, D. L., MOCHIZUKI, D. Y., HENNEY, C. S., AND GILLIS, S.: Interleukin 2 administered in vivo induces the growth of cultured T cells in vivo. J. Immunol. **132:** 2259–2265, 1984a.

CHEEVER, M. A., THOMPSON, J. A., KERN, D. E., AND GREENBERG, P. D.: Interleukin-2 administered in vivo induces the growth and augments the function of cultured T cells in vivo. J. Biol. Response Modif. **3:** 462–467, 1984b.

CHEEVER, M. A., THOMPSON, J. A., KERN, D. E., AND GREENBERG, P. D.: Interleukin 2 (IL 2) administered in vivo: influence of IL 2 route and timing on T cell growth. J. Immunol. **134:** 3895–3900, 1985b.

CHEN, B. D., AND NAJOR, F.: Macrophage activation by interferon alpha and beta is associated with a loss of proliferative capacity: role of interferon alpha and beta in the regulation of macrophage proliferation and function. Cell. Immunol. **106:** 343–354, 1987.

CHONG, K. T.: Prophylactic administration of interleukin-2 protects mice from lethal challenge with Gram-negative bacteria. Infect. Immun. **55:** 668–673, 1987.

CHONG, K. T., AND HUSTON, M.: The implications of endotoxin contamination in the evaluation of anti-LPS antibodies against Gram-negative sepsis. J. Infect. Dis. **156:** 713–719, 1987.

CHOROMANSKI, L., AND KUHN, R. E.: Interleukin 2 enhances specific and nonspecific immune responses in experimental Chagas' disease. Infect. Immun. **50:** 354–357, 1985.

CHOROMANSKI, L., AND KUHN, R. E.: Use of parasite antigens and interleukin-2 to enhance suppressed immune responses during *Trypanosoma cruzi* infection in mice. Infect. Immun. **55:** 403–408, 1987.

CLINTON, B. A., ORTIZ-ORTIZ, L., GARCIA, W., MARTINEZ, T., AND CAPIN, R.: *Trypanosoma cruzi:* early immune responses in infected mice. Exp. Parasitol. **37:** 417–425, 1975.

COHEN, F. E., KOSEN, P. A., KUNTZ, I. D., EPSTEIN, L. B., CLARDELLI, T. L., AND SMITH, K. A.: Structure-activity studies of interleukin-2. Science (Wash. DC) **234:** 349–352, 1986.

COLIZZI, V.: In vivo and in vitro administration of interleukin 2-containing preparation reverses T-cell unresponsiveness in *Mycobacterium bovis* BCG-infected mice. Infect. Immun. **45:** 25–28, 1984.

COLLINS, F. M., AND WATSON, S. R.: Suppressor T cells in BCG-infected mice. Infect. Immun. **25:** 491–496, 1979.

COLLINS, F. M., MORRISON, N. E., AND MANTALBINE, V.: Immune response to persistent mycobacterial infection in mice. Infect. Immun. **20:** 430–438, 1978.

COLLINS, T., LAPIERRRE, L. A., FIERS, W., STROMINGER, J. L., AND POBER, J. S.: Recombinant human tumor necrosis factor increases mRNA levels and surface expression of HLA-A, B antigens in vascular endothelial cells and dermal fibroblasts in vitro. Proc. Natl. Acad. Sci. USA **83:** 446–450, 1986.

CONLON, P. J., HEFENEIDER, S. H., HENNEY, C. S., AND GILLIS, S.: The effects of interleukin 2 on primary in vivo immune responses. *In* The Potential Role of T cells in Cancer Therapy, ed by A. Fefer and A. Goldstein, pp. 113–125, Raven Press, New York, 1982.

CONLON, P. J., WASHKEWICZ, T. L., MOCHIZUKI, D. Y., URDAL, D. L., GILLIS, S., AND HENNEY, C. S.: The treatment of induced immune deficiency with interleukin-2. Immunol. Lett. **10:** 307–314, 1985.

CORBETT, T. H., ROBERTS, B. J., LEOPOLD, W. R., PECKHAM, J. C., WILKOFF, L. J., GRISWOLD D. P., AND SCHABEL, F. M.: Induction and chemotherapeutic response of two transplantable adenocarcinomas of the pancreas in C57Bl/6 mice. Cancer Res. **44:** 717–726, 1984.

DAMLE, N. K., DOYLE, L. V., AND BRADLEY, E. C.: IL-2-activated human killer cells are derived from phenotypically-heterogeneous precursors. J. Immunol. **137:** 2814–2822, 1986.

DAMLE, N. K., DOYLE, L. V., BENDER, J. R., AND BRADLEY, E. C.: Interleukin 2-activated human lymphocytes exhibit enhanced adhesion to normal vascular endothelial cells and cause their lysis. J. Immunol. **138:** 1779–1785, 1987.

DAVIS, F. F., ABUCHOWSKI, A., VAN ES, T., PALCZUK, N. C., SAVOCA, K., CHEN, R. H., AND PYATAK, P.: Soluble, nonantigenic polyethylene glycol-bound enzymes. *In* BioMedical Polymers, pp. 441–451, Academic Press, New York, 1980.

DENICOFF, S. A., AND RUBINOW, D. R.: The neuroendocrine effects of interleukin-2 treatment. J. Clin. Endocrinol. Metab. **69:** 402–410, 1989.

DEVOS, R., PLAETINCK, G., CHEROUTRE, H., SIMONS, G., DEGRAVE, W., TAVERNIER, J., REMAUT, E., AND FIERS. W.: Molecular cloning of human interleukin 2 cDNA and its expression on *E. coli.* Nucleic Acids Res. **11:** 4307–4323, 1983.

DONOHUE, J. H., AND ROSENBERG, S. A.: The fate of interleukin 2 after in vivo administration. J. Immunol. **130:** 2203–2208, 1983.

DONOHUE, J. H., LOTZE, M. T., ROBB, R. J., ROSENSTEIN, M. BRAZIEL, R. M., JAFFE, E. S., AND ROSENBERG, S. A.: *In vivo* administration of purified Jurkat-derived interleukin 2 in mice. Cancer Res. **44:** 1380–1386, 1984b.

DONOHUE, J. H., ROSENSTEIN, M., CHANGE, A. E., LOTZE, M. T., ROBB, R. J., AND ROSENBERG, S. A.: The systemic administration of purified interleukin 2 enhances the ability of sensitized murine lymphocytes to cure a disseminated syngeneic lymphoma. J. Immunol. **132:** 2123–2128, 1984a.

EBERLEIN, T. J., ROSENSTEIN, M., AND ROSENBERG, S. A.: Regression of a disseminated syngeneic solid tumor by systemic transfer of lymphoid cells expanded in interleukin 2. J. Exp. Med. **156:** 385–397, 1982.

EGGERMONT, A. M., AND SUGARBAKER, P. H.: Lymphokine-activated killer cell and IL-2 inhibitors: their role in adoptive immunotherapy. Cell. Immunol. **107:** 384–394, 1987.

EGGERMONT, A. M., STELLER, E. P., OTTOW, R. T., MATTHEWS, W., AND SUGARBAKER, P. H.: Augmentation of interleukin-2 immunotherapeutic effects by lymphokine-activated killer cells and allogeneic stimulation in murine tumor cells. J. Natl. Cancer Inst. **79:** 983–990, 1987.

EGGERMONT, A. M., SUGARBAKER, P. H., MARQUET, R. L., AND JEEKEL, J.: In vivo generation of LAK cell activity by ABPP and rIL-2 and their anti-tumor effects against immunogenic and nonimmunogenic tumors in murine models. Cancer Immunol. Immunother. **26:** 23–30, 1988.

ELLISON, M. D., POVLISHOCK, J. T., AND MERCHANT, R. E.: Blood-brain barrier dysfunction in cats following rIL-2 infusion. Cancer Res. **47:** 5765–5770, 1987.

ESTROV, A., ROIFMAN, C., MILLS, G., GRUNBERGER, T., GELFAND, E. W., AND FREEDMAN, M. H.: The regulatory role of interleukin 2-responsive T lymphocytes on human marrow granulopoiesis. Blood **69:** 1161–1166, 1987.

ETTINGHAUSEN, S. E., AND ROSENBERG, S. A.: Immunotherapy of murine sarcomas using lymphokine activated killer cells: optimization of the schedule and route of administration of recombinant interleukin-2. Cancer Res. **46:** 2784–2792, 1986.

ETTINGHAUSEN, S. E., LIPFORD, E. H., MULE, J. J., AND ROSENBERG, S A.: Recombinant interleukin 2 stimulates in vivo proliferation of adoptively transferred lymphokine-activated killer (LAK) cells. J. Immunol. **135:** 3623–3635, 1985.

EVANS, R.: Failure to relate the anti-tumor action of cyclophosphamide with immunogenicity of two murine fibrosarcomas. Int. J. Cancer **21:** 611–616, 1978.

EVANS, R.: Combination therapy by using cyclophosphamide and tumor-sensitized lymphocytes: a possible mechanism of action. J. Immunol. **130:** 2511–2513, 1983.

FAHEY, J. R., AND HINES, D. L.: Progressive growth of immunogenic tumors: relationship between susceptibility of ascites P815 tumor cells to T-cell-mediated lysis and immune destruction *in vivo.* Cancer Res. **47:** 4759–4765, 1987.

FAIRMAN, R. P., GLAUSER, F. L., MERCHANT, R. E., BECHARD, D., AND FOWLER, A. A.: Increase of rat pulmonary microvascular permeability to albumin by rIL-2. Cancer Res. **47:** 3528–3532, 1987.

FARRAR, W. L.: Endorphin modulation of lymphokine activity. *In* Opioid Peptides in the Periphery, ed by F. Fraioli, A. Isidori, and M. Mazzeti, pp. 159–165, Elsevier, New York, 1984.

FIDLER, I. J.: Selection of successive tumour lines for metastasis. Nature New Biol. **242:** 148–149, 1973.

FODSTAD, O., HANSEN, C. T., CANNON, G. B., STATHAM, C. N., LICHTENSTEIN, G. R., AND BOYD, M. R.: Lack of correlation between natural killer activity and tumor growth control in nude mice with different immune defects. Cancer Res. **44:** 4403–4406, 1984.

FORNI, G., GIOVARELLI, M., AND SANTONI, A.: Lymphokine-activated tumor inhiibition in vivo. I. The local administration of interleukin-2 triggers non-reactive lymphocytes from tumor-bearing mice to inhibit tumor growth. J. Immunol. **134:** 1305–1311, 1985.

FUJITA, T., TAKAOKA, C., MATSUI, H., AND TANIGUCHI, T.: Structure of the human interleukin 2 gene. Proc. Natl. Acad. Sci. USA **80:** 7437–7441, 1983.

GALLATIN, W. M., WEISSMAN, I. L., AND BUTCHER, E. C.: A cell-surface molecule involved in organ-specific homing of lymphocytes. Nature (Lond.) **304:** 30–34, 1983.

GARZON, F. T., SALAS, M., BERGER, M. R., AND KIRCHNER, H.: Effect of interleukin-2 on the manifestation and growth of acetoxymethyl-methylnitrosamine-induced colorectal rat adenocarcinoma. J. Cancer Res. Clin. Oncol. **111:** 79–81, 1986.

GATELY, M. K., ANDERSON, T. D., AND HAYES, T. J.: Role of asialo-GM1-positive lymphoid cells in mediating the toxic effects of recombinant rIL-2 in mice. J. Immunol. **141:** 189–200, 1988.

GEARING, A. J. H., WADHWA, M., AND PERRIS, A. D.: In vivo administration of interleukin 2 stimulates mitosis in thymus and bone marrow. Eur. J. Immunol. **16:** 1171–1174, 1986.

GECZY, C. L.: The role of lymphokines in delayed-type hypersensitivity reactions. Springer Semin. Immunopathol. **7:** 321–346, 1984.

GLAUSER, F. L., DEBOIS, G. G., BECHARD, D. E., MERCHANT, R. E., GRANT, A. J., FOWLER, A. A., AND FAIRMAN, R. P.: Cardiopulmonary effects of rIL-2 infusion in sheep. J. Appl. Physiol. **64:** 1030–1037, 1988.

GOODMAN, M. G.: Interaction between cytokines and 8-mercaptoguanosine in humoral immunity: synergy with interferon. J. Immunol. **139:** 142–146, 1987.

GREENBERG, P. D., CHEEVER, M A., AND FEFER, A.: H-2 restriction of adoptive immunotherapy of advanced tumors. J. Immunol. **126:** 2100–2103, 1981.

GREGG, E. O., GREGG, I. C., AND GREEN, I.: Generation of lymphokine-activated killer cells in strain 2 guinea pigs and their use in the therapy of L2C, and acute B-cell leukemia. Cancer Res. **47:** 723–729, 1987.

GRESSER, I., AND BOURALI, C.: Antitumor effects of interferon preparations in mice. J. Natl. Cancer Inst. **45:** 365–376, 1970.

GRIMM, E. A., AND ROSENBERG, S. A.: The human lymphokine-activated killer cell phenomenon. *In* Lymphokines, vol. 9, ed. by E. Pick, pp. 279–311, Academic Press, New York, 1984.

GRIMM, E. A., RAMSEY, K. M., MAZUMDER, A., WILSON, D. J., DJEU, J. Y., AND ROSENBERG, S. A.: Lymphokine-activated killer (LAK) cell phenomenon. II. The precursor phenotype is serologically distinct from peripheral T lymphocytes, memory CTL, and NK cells. J. Exp. Med. **157:** 884–897, 1983a.

GRIMM, E. A., ROBB, R. J., ROTH, J. A., NECKERS, L. M., LACHMAN, L. B., WILSON, D. J., AND ROSENBERG, S. A.: Lymphokine-activated killer (LAK) cell phenomenon. III. Evidence that rIL-2 is sufficient for direct activation of PBL into LAK. J. Exp. Med. **158:** 1356–1361, 1983b.

HALL, J. G.: Effect of skin with oxazolone on the local extravasation of mononuclear cells in sheep. Ciba Found. Symp. **71:** 197–209, 1980.

HALLER, O., KIESSLING, R., ORN, A., AND WIGZELL, H.: Generation of natural killer cells: an autonomous function of the bone marrow. J. Exp. Med. **145:** 1411–1416, 1977.

HANDA, K., SUZUKI, R., MATSUI, H., SHIMIZU, Y., AND KUMAGAI, K.: Natural killer (NK) cells as a responder to interleukin-2 (rIL-2). II. rIL-2-induced interferon-gamma production. J. Immunol. **130:** 988–992, 1983.

HARDT, C., ROLLINGHOFF, M., PFIZENMAIER, K., MOSMANN, H., AND WAGNER, H.: Lyt-23+ cyclophosphamide-sensitive T cells regulate the activity of an interleukin 2 inhibitor in vivo. J. Exp. Med. **154:** 262–274, 1981.

HARLAND, J. M.: Leukocyte-endothelial interactions. Blood **65:** 513–525, 1985.

HARLEN-BELLAN, A., JOSKOWICZ, M., FRADELIZI, D., AND EISEN, H.: Modification of T-cell proliferation and interleukin 2 production in mice infected with *Trypanosoma cruzi.* Proc. Natl. Acad. Sci. USA **80:** 3466–3469, 1983.

HAY, E. D. (ed.).: Cell Biology of Extracellular Matrix, Plenum Publishing Corp., New York, 1981.

HEFENEIDER, S., CONLON, P., HENNEY, C., AND GILLIS, S.: In vivo interleukin

2 administration augments the generation of alloreactive cytolytic T lymphocytes and resident natural killer cells. J. Immunol. **130:** 222–227, 1983.

HENNEY, C. S., KURIBAYASHI, K., KERN, D. E., AND GILLIS, S.: Interleukin-2 augments natural killer activity. Nature (Lond.) **291:** 335–338, 1981.

HERECOURT, J., AND RICHET, C.: Self recognition in cancer. Nehal. Searc. Acad. Sci. **121:** 567–573, 1895.

HERON, I., HOKLAND, M., AND BERG, K.: Enhanced expression of beta$_2$-microglobulin and HLA antigens on human lymphoid cells by interferon. Proc. Natl. Acad. Sci. USA **75:** 6215–6219, 1978.

HICKS, N. J., MORRIS, A. G., AND BURKE, D. C.: Partial reversion of the transformed phenotype of murine sarcoma virus-transformed cells in the presence of interferon: a possible mechanism for the antitumour effect of interferon. J. Cell Sci. **49:** 225–236, 1981.

HOFFENBACH, A., LAGRANCE, P. H., AND BACH, M. A.: Deficit of interleukin 2 production associated with impaired T-cell proliferative responses in *Mycobacterium lepraemurium* infection. Infect. Immun. **39:** 109–116, 1983.

HOLTER, W., GRUNOW, R., STOCKINGER, H., AND KNAPP, W.: Recombinant IFN-gamma induces rIL-2 receptors on human peripheral blood monocytes. J. Immunol. **136:** 2171–2175, 1986.

HURLEY, J. V.: An electron microscopic study of leukocytic emigration and vascular permeability in rat skin. Aust. J. Exp. Biol. **41:** 171–186, 1963.

HURLEY, J. V.: Inflammation. *In* Edema, ed by N. C. Staub and A. E. Taylor, pp. 463–488, Raven Press, New York, 1984.

IIGO, M., SAKURAI, M., TAMURA, T., SAIJO, N., AND HOSHI, A.: *In vivo* antitumor activity of multiple injections of recombinant interleukin 2, alone and in combination with three different types of recombinant interferon, on various syngenei murine tumors. Cancer Res. **48:** 260–264, 1988.

IIZAWA, Y., NISHI, T., KONDO, M., TSUCHIYA, K., AND IMADA, A.: Effect of recombinant human interleukin-2 on the course of experimental chronic respiratory tract infection caused by Klebsiella pneumoniae in mice. Infect. Immun. **56:** 45–50, 1988.

INDROVA, M., BUBENIK, J., AND TOULCOVA, A.: Immunotherapy of murine sarcomas with interleukin 2. II. Activation of killer cells by human recombinant rIL-2. Folia Biol. (Praha) **32:** 392–397, 1986.

ISHIDA, Y., NISHI, M., TAGUCHI, O., INABA, K., HATTORI, M., MINATO, N., KAWAICHI, M., AND HONJO, T.: Expansion of natural killer cells but not T cells in human interleukin 2/interleukin 2 receptor (Tac) transgenic mice. J. Exp. Med. **170:** 1103–1115, 1989a.

ISHIDA, Y., NISHI, M., TAGUCHI, O., INABA, K., MINATO, N., KAWAICHI, M., AND HONJO, T.: Effects of the deregulated expression of human interleukin-2 in transgenic mice. Int. Immunol. **1:** 113, 1989b.

ISHIZAKA, S. T., AND STUTMAN, O.: Analysis by limiting dilution of interleukin 2-producing T cells in murine ontogeny. Eur. J. Immunol. **13:** 936–942, 1983.

JAIN, R. K.: Transport of molecules in the tumor interstitium: a review. Cancer Res. **47:** 3039–3051, 1987.

JEEVAN, A., AND ASHERSON, G. L.: Recombinant interleukin-2 limits the replication of *Mycobacterium lepraemurium* and *Mycobacterium bovis* BCG in mice. Infect. Immun. **56:** 660–664, 1988.

JELSEMA, C. L., KILLION, J. J., AND WINKELHAKE, J. L.: Enzymatic alteration of cell-surface antigenicity. *In* Enzymes as Drugs, ed by J. C. Holcenberg, pp. 259–312, John Wiley and Sons, New York, 1981.

JENKINSON, E. J., KINGSTON, R., AND OWEN, J. J. T.: Importance of IL-2 receptors in intrathymic generation of cells expressing T-cell receptors. Nature (Lond.) **329:** 160–162, 1987.

JESMOK, G. J., AND GUNTHER, R. A.: In vivo biology of recombinant interleukin-2 infusion in sheep: cardiopulmonary manifestations of an intravascular immune-inflammatory response. Inflammation **13:** 267–284, 1989.

KAHAN, D. B., NOMI, S., AND PELLIS, N. R.: Specific chemoimmunotherapy in tumor-bearing mice with extracted antigen, cyclophosphamide, and intrasplenic administration of interleukin-2. Cancer (Phila.) **57:** 477–483, 1986.

KATAOKA, T., MATSUURA, N., OH-HASHI, F., AND SUHARA, Y.: Treatment regimen and host T-cell-dependent therapeutic effect of interferon in mouse solid tumors. Cancer Res. **45:** 3548–3553, 1985.

KATRE, N. V., KNAUF, M. J., AND LAIRAD, W. L.: Chemical modification of recombinant interleukin 2 by polyethylene glycol increases its potency in the mlurine Meth A sarcoma model. Proc. Natl. Acad. Sci. USA **84:** 1487–1491, 1987.

KAWAMURA, H., ROSENBERG, S. A., AND BERZOFSKY J. A.: Immunization with antigen and interleukin 2 in vivo overcomes Ir gene low responsiveness. J. Exp. Med. **162:** 381–386, 1985.

KEDAR, E., IKEJIRI, B. I., BONNARD, G. D., AND HERBERMAN, R. B.: A rapid technique for isolation of viable tumor cells from solid tumors: use of the tumor cells for induction and measurement of cell-mediated cytotoxic responses. Eur. J. Clin. Oncol. **18:** 991–1000, 1982.

KELLEY, V. E., GAULTON, G. N., HATTORI, M., IKEGAMI, H., EISENBARTH, G., AND STROM, T. B.: Anti-interleukin 2 receptor antibody suppresses murine diabetic insulitis and lupus nepharitis. J. Immunol. **140:** 59–61, 1988.

KEMPF, R. A., AND MITCHELL, M. S.: Effects of chemotherapeutic agents on the immune response I. Cancer Invest. **2:** 459–66, 1984.

KEMPF, R. A., AND MITHCELL, M. S.: Effects of chemotherapeutic agents on the immune response II. Cancer Invest. **3:** 23–33, 1985.

KIRKMAN, R. L., BARRETT, L. V., GAULTON, G. N., KELLEY, V. E., YTHIER, A., AND STROM, T. B.: Administration of an anti-interleukin 2 receptor monoclonal antibody prolongs cardiac allograft survival in mice. J. Exp. Med. **162:** 358–362, 1985.

KLEIN, G.: Tumor antigens. Annu. Rev. Microbiol. **20:** 223–252, 1966.

KLEINERMAN, E. S., ZWELLING, L. A., AND MUCHMORE, A. V.: Enhancement of naturally occuring human spontaneous monocyte-mediated cytotoxicity by *cis*-diamminedichloroplatinum(II). Cancer Res. **40:** 3099–3102, 1980.

KNOP, J., TABORSKI, B., AND DEMAEYER-GUIGNARD, J.: Selective inhibition of the generation of T suppressor cells of contact sensitivity in vitro by interferon. J. Immunol. **138:** 3684–3687, 1987.

KOTHS, K., AND HALENBECK, R.: Pharmacokinetic studies on 35S-labeled recombinant interleukin-2 in mice. *In* Cellular and Molecular Biology of Lymphokines, ed. by C. Sorg and A. Schimpl, pp. 779–783, Academic Press, New York, 1985.

KOZUMBO, W. J., HARRIS, D. T., GROMKOWSKI, S., CEROTTINI, J.-C., AND CERUTTI, P. A.: Molecular mechanisms involved in *T* cell activation. II. The phosphatidylinositol signal transducing mechanism. J. Immunol. **138:** 606–612, 1987.

LAFRENIERE, R., AND ROSENBERG, S. A.: Adoptive immunotherapy of murine hepatic metastases with lymphokine activated killer (LAK) cells and recombinant interleukin 2 (RIL 2) can mediate the regression of both immunogenic and nonimmunogenic sarcomas and an adenocarcinoma. J. Immunol. **135:** 4273–4280, 1985a.

LAFRENIERE, R. R., AND ROSENBERG, S. A.: Successful immunotherapy of murine experimental hepatic metastases with lymphokine-activated killer cells and recombinant interleukin-2. Cancer Res. **45:** 3735–3741, 1985b.

LALA, P. K., AND PARHAR, R. S.: Cure of B16F10 melanoma lung metastasis in mice by chronic indomethacin therapy combined with repeated rounds of interleukin 2: characteristics of killer cells generated *in situ.* Cancer Res. **48:** 1072–1079, 1988.

LALA, P. K., SANTER, V., LIBENSON, H., AND PARHAR, R. S.: Changes in the host NK cell population in mice during tumor development. I. Kinetics and in vivo significance. Cell. Immunol. **93:** 250–264, 1985.

LAMOUREUX, G., AND POISSON, R.: BCG and immunological anergy. Lancet **2:** 989–991, 1974.

LANDSTEINER, K., AND CHASE, M. W.: Experiments on transfer of cutaneous sensitivity to simple compounds. Proc. Soc. Exp. Biol. Med. **49:** 688–695, 1942.

LAROYE, G. J.: Cancer caused by an inherited selective defect in immunological surveillance. Lancet **1:** 641–643, 1973.

LAUWERYNS, J. M.: The blood and lymphatic microcirculation of the lung. Pathobiol. Annu. **6:** 365–415, 1971.

LEFEVER, A. V., TRUITT, R. L., SHIH, C. C-Y., LIEPINS, A., AND BORTIN, M. M.: Migration patterns and functional activity of cloned cytotoxic T lymphocytes in syngeneic and allogeneic mice. Transplantation (Baltimore) **37:** 410–417, 1984.

LEWINSOHN, D. M., BARGATZE, R. F., AND BUTCHER, E. C.: Leukocyte-endothelial cell recognition: evidence of a common molecular mechanism shared by neutrophils, lymphocytes, and other leukocytes. J. Immunol. **138:** 4313–4321, 1987.

LOTZE, M. T., GRIMM, E. A., MAZUMDER, A., STRAUSSER, J. L., AND ROSENBERG, S. A.: Lysis of fresh and cultured autologous tumor by human lymphocytes cultured in T cell growth factor (TCGF). Cancer Res. **41:** 4420–4425, 1981.

LOTZE, M. T., MATORY, Y. L., ETTINGHAUSEN, S. E. RAYNER, A. A., SHARROW, S. O., SEIPP, C. Y., CUSTER, M. C., AND ROSENBERG, S. A.: In vivo administration of purified human interleukin 2. II. Half life, immunologic effects, and expansion of peripheral lymphoid cells in vivo with recombinant IL 2. J. Immunol. **135:** 2865–2875, 1985.

LOTZE, M. T., MATORY, Y. L., RAYNER, A. A., ETTINGHAUSEN, S. E., VETTO, J. T., SEIPP, C. A., AND ROSENBERG, S. A.: Clinical effects and toxicity of interleukin 2 in patients with cancer. Cancer (Phila.) **58:** 2764–2772, 1986.

MAKINODAN, T., AND KAY, M.: Age influence on the immune system. Adv. Immunol. **29:** 287–330, 1980.

MALKOVSKY, M., AND MEDAWAR, P. B.: Is immunological tolerance (non-responsive) a consequence of interleukin 2 deficit during the recognition of antigen? Immunol. Today **5:** 340–343, 1984.

MALKOVSKY, M., BRENNER, M. K., HUNT, R., RASTAN, S., DORE, C., BROWN, S., NORTH, M. E., ASHERSON, G. L., PRENTICE, H. G., AND MEDAWAR, P. B.: T-cell depletion of allogeneic bone marrow prevents acceleration of graft-versus-host disease induced by exogenous interleukin 2. Cell. Immunol. **103:** 476–480, 1986.

MALKOVSKY, M., LOVELAND, B., NORTH, M., ASHERSON, G. L., GAO, L., WARD, P., AND FIERS, W.: Recombinant interleukin-2 directly augments the cytotoxicity of human monocytes. Nature (Lond.) **325:** 262–265, 1987.

MALKOVSKY, M., MEDAWAR, P. B., THATCHER, D. R., TOY, J., HUNT, R., RAYFIELD, L. S., AND DORE, C.: Acquired immunological of foreign cells is impaired by recombinant interleukin 2 or vitamin A acetate. Proc. Natl. Acad. Sci. USA **82:** 536–538, 1985.

MARK, D. F., DOYLE, M. Y., AND KOTHS, K.: Human interleukin-2. *In* Recombinant lymphokines and their receptors, edited by S. Gillis, pp. 1–31, Marcel Dekker, Inc., New York, 1987.

MARKMAN, M.: Intracavitary administration of biological agents. J. Biol. Response Modif. **6:** 404–411, 1987.

MARKMAN, M.: Intracavitary chemotherapy. CRC Crit. Rev. Oncol/Hemotol. **3:** 205–233, 1985.

MATIS, L. A., SHU, S., GROVES, E. S., ZINN, S., CHOU, T., KRUISBEEK, A. M., ROSENSTEIN, M., AND ROSENBERG, S. A.: Adoptive immunotherapy of a syngeneic murine leukemia with a tumor-specific cytotoxic T cell clone and

recombinant human interleukin 2: correlation with clonal IL 2 receptor expression. J. Immunol. **136:** 3496–3501, 1986.

MATORY, Y. L., CHANG, A. E., LIPFORD, E. H., BRAZIEL, R., HYATT, C. L., MCDONALD, H. D., AND ROSENBERG, S. A.: Toxicity of recombinant human interleukin-2 in rats following intravenous infusion. J. Biol. Response Modif. **4:** 377–390, 1985.

MAZUMDER, A., AND ROSENBERG, S. A.: Successful immunotherapy of natural killer-resistant established pulmonary melanoma metastases by the intravenous adoptive transfer of syngeneic lymphocytes activated in vitro by interleukin 2. J. Exp. Med. **159:** 495–507, 1984b.

MAZUMDER, A., EBERLEIN, T. J., GRIMM, E. A., WILSON, D. J., KEENAN, A. M., AAMODT, R., AND ROSENBERG, S. A.: Phase I study of the adoptive immunotherapy of human cancer with lectin activated autologous mononuclear cells. Cancer (Phila.) **53:** 896–905, 1984a.

MCINTOSH, J. K., MULE, J. J., MERINO, M. J., AND ROSENBERG, S. A.: Synergistic antitumor effects of immunotherapy with recombinant interleukin-2 and recombinant tumor necrosis factor-alpha. Cancer Res. **48:** 4011–4017, 1988.

METCALF, D.: The molecular biology and functions of the granulocyte-macrophage colony-stimulating factors. Blood **67:** 257–267, 1986.

MILLER, R. G.: Immune suppression by clonal deletion. *In* Strategies of Immune Regulation, ed. by E. Sercarz and A. Cunningham, p. 507, Academic Press, New York, 1980.

MILLS, G. B., CARLSON, G., AND PAETKAU, V.: Generation of cytotoxic lymphocytes to syngeneic tumors by using co-stimulator (interleukin 2): in vivo activity. J. Immunol. **125:** 1904–1909, 1980.

MILLS, G. B., GIRARD, P., GRINSTEIN, S., AND GELFAND, E. W.: Interleukin-2 induces proliferation of T-lymphocyte mutants lacking protein kinase C. Cell **55:** 91–100, 1988.

MITCHELL, M. S.: Combining chemotherapy with biological response modifiers in treatment of cancer. J. Natl. Cancer Inst. **80:** 1445–1450, 1988.

MOKYR, M. B., AND DRAY, S.: Some advantages of curing mice bearing a large subcutaneous MOPC-315 tumor with a low dose rather than a high dose of cyclophosphamide. Cancer Res. **43:** 3112–3119, 1983.

MOKYR, M. B., BRAUN, D. P., AND DRAY, S.: Augmentation of antitumor cytotoxicity in MOPC-315 tumor-bearer spleen cells by depletion of glass-adherent cells prior to *in vitro* activation. Cancer Res. **39:** 785–792, 1979.

MORGAN, D. A., RUSCETTI, F. W., AND GALLO, R.: Selective in vitro growth of T lymphocytes from normal human bone marrow. Science (Wash. DC) **193:** 1007–1008, 1976.

MORIKAWA, K., OKADA, F., HOSOKAWA, M., AND KOBAYASHI, H.: Enhancement of therapeutic effects of recombinant interleukin 2 on a transplantable rat fibrosarcoma by the use of a sustained release vehicle, pluronic gel. Cancer Res. **47:** 37–41, 1987.

MULE, J. J., ETTINGHAUSEN, S. E., SPIESS, P. J., SHU, S., AND ROSENBERG, S. A.: Antitumor efficacy of lymphokine-activated killer cells and recombinant rIL-2 *in vivo:* survival benefit and mechanisms of tumor escape in mice undergoing immunotherapy. Cancer Res. **46:** 676–683, 1986a.

MULE, J. J., SHU, S., AND ROSENBERG, S. A.: The anti-tumor efficacy of lymphokine-activated killer cells and recombinant interleukin 2 in vivo. J. Immunol. **135:** 646–652, 1985.

MULE, J. J., SHU, S., SCHWARZ, S. L., AND ROSENBERG, S.: Adoptive immunotherapy of established pulmonary metastases with LAK cells and recombinant interleukin-2. Science (Wash. DC) **225:** 1487–1489, 1984.

MULE, J. J., YANG, J., SHU, S., AND ROSENBERG, S. A.: The anti-tumor efficacy of lymphokine-activated killer cells and recombinant interleukin 2 in vivo: direct correlation between reduction of established metastases and cytolytic activity of lymphokine-activated killer cells. J. Immunol. **136:** 3899–3909, 1986b.

MURPHY, G. P., AND HRUSHESKY, W. J.: A murine renal cell carcinoma. J. Natl. Cancer Inst. **50:** 1013–1025, 1973.

MURRAY, J. L., AND KOLLMORGEN, G. M.: Inhibition of lymphocyte response by prostaglandin-producing suppressor cells in patients with melanoma. J. Clin. Immunol. **3:** 268–76, 1983.

NAKAMURA, R. M., AND TOKUNAGA, T.: Induction of suppressor T cells in delayed-type hypersensitivity to *Mycobacterium bovis* BCG in low-responder mice. Infect. Immun. **28:** 331–335, 1980.

NEDWIN, G. E., SVEDERSKY, L. P., BRINGMAN, T. S., PALLADINO, M. A., AND GOEDDEL, D. V.: Effect of interleukin 2, interferon-gamma, and mitogens on the production of tumor necrosis factors alpha and beta. J. Immunol. **135:** 2492–2497, 1985.

NETA, R., VOGEL, S. N., OPPENHEIM, J. J., AND DOUCHES, S. D.: Cytokines in radioprotection: comparison of the radioprotective effects of IL-1 to rIL-2, GM-CSF and IFN-gamma. Lymphokine Res. **5:** S105–S110, 1986.

NISHI, M., ISHIDA, Y., AND HONJO, T.: Expression of functional interleukin-2 receptors in human light/Tac transgenic mice. Nature (Lond.) **331:** 267, 1988.

NISHIMURA, T., OHTA, S., SATO, N., TOGASHI, Y., GOTO, M., AND HASHIMOTO, Y.: Combination tumor-immunotherapy with recombinant tumor necrosis factor and recombinant interleukin 2 min mice. Int. J. Cancer **40:** 255–261, 1987.

NISHIMURA, T., TOGASHI, Y. GOTTO, M., YAGI, H., UCHIYAMA, Y., AND HASHIMOTO, Y.: Augmentation of the therapeutic efficacy of adoptive tumor immunotherapy by in vivo administration of slowly released recombinant interleukin 2. Cancer Immunol. Immunother. **21:** 12–18, 1986a.

NISHIMURA, T., UCHIYAMA, Y. YAGI, H., AND HASHIMOTO, Y.: Administration of slowly released recombinant interleukin 2: augmentation of the efficacy of adoptive immunotherapy with lymphokine-activated killer (LAK) cells. J. Immunol. Methods **91:** 21–27, 1986b.

NOMI, S., NAITO, K., KAHAN, B. D., AND PELLIS, N. R.: Intrasplenic administration of interleukin-2 to potentiate specific chemoimmunotherapy in tumor-bearing mice. Cancer Res. **46:** 5606–5610, 1986.

NOMI, S., PELLIS, N. R., AND KAHAN, B. D.:Retardation of postsurgical metastases with the use of extracted tumor-specific transplantation antigens and cyclophosphamide. J. Natl. Cancer Inst. **73:** 943–950, 1984.

NORTH, R. J.: Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. J. Exp. Med. **55:** 1063–1074, 1982.

NORTH, R. J.: Down-regulation of the antitumor immune response. Adv. Cancer Res. **45:** 1–43, 1985.

NOSSAL, G. J. V.: Cellular mechanisms of immunologic tolerance. Annu. Rev. Immunol. **1:** 33–62, 1983.

NOSSAL, G. J. V.: Immunologic tolerance: collaboration between antigen and lymphokines. Science (Wash. DC). **245:** 147–153, 1989.

NOWELL, P. C.: The clonal evolution of tumor cell populations. Science (Wash. DC) **194:** 23–28, 1976.

NUNBERG, J. H., DOYLE, M. V., YORK, S. M., AND YORK, C. J.: Interleukin 2 acts as an adjuvant to increase the potency of inactivated rabies virus vaccine. Proc. Natl. Acad. Sci. USA **86:** 4240–4243, 1989.

OKUSAWA, S., YANCEY, K. B., VAN DER MEER, J. W. M., ENDRES, S., LONNEMANN, G., HEFTER, K., FRANK, M. M., BURKE, J. F., DINARELLO, C. A., AND GELFAND, J. A.: C5a stimulates secretion of tumor necrosis factor from human mononuclear cells in vitro. J. Exp. Med. **168:** 443–448, 1988.

OLD, L. J., BENACERRAF, B., CLARKE, D. A., CARSWELL, E. A., AND STOCKERT, E.: The role of the reticuloendothelial system in the host reaction to neoplasia. Cancer Res. **21:** 1281–1301, 1961.

ORTALDO, J. R., PORTER, H. R., MILLER, P., STEVENSON, H. C., OZOLS, R. F., AND HAMILTON, T. C.: Adoptive cellular immunotherapy of human ovarian carcinoma xenographs in nude mice. Cancer Res. **46:** 4414–4419, 1986.

OSAWA, H., JOSIMOVIC-ALASEVIC, O., AND DIAMATSTEIN, T.: Interleukin 2 receptors are released by cells in vitro and in vivo. I. Detection of soluble IL2 receptors in cell culture supernatants and in the serum of mice by an immunoradiometric assay. Eur. J. Immunol. **16:** 467–469, 1986.

OTTOW, R. T., STELLER, E. P., SUGARBAKER, P. H., WESLEY, R. A., AND ROSENBERG, S. A.: Immunotherapy of intraperitoneal cancer with interleukin 2 and lymphokine-activated killer cells reduces tumor load and prolongs survival in murine models. Cell. Immunol. **104:** 366–376, 1987.

PALLADINO, M. A., WELTE, K., CARROLL, A. M. AND OETTGEN, H. F.: Characterization of interleukin 2 (rIL-2)-dependent cytotoxic T-cell clones. V. Transfer of resistance to allografts and tumor grafts requires exogenous rIL-2. Cell. Immunol. **86:** 299–307, 1984.

PAPA, M. Z., MULE, J. J., AND ROSENBERG, S. A.: Antitumor efficacy of lymphokine-activated killer cells and recombinant interleukin 2 in vivo: successful immunotherapy of established pulmonary metastases from weakly immunogenic and nonimmunogenic murine tumors of three distinct histological types. Cancer Res. **46:** 4973–4978, 1986b.

PAPA, M. Z., VETTO, J. T., ETTINGHAUSEN, S. E., MULE, J. J., AND ROSENBERG, S. A.: Effect of corticosteroid on the antitumor activity of lymphokine-activated killer cells and interleukin 2 in mice. Cancer Res. **46:** 5618–5623, 1986a.

PAPA, M. Z., YANG, J. C., VETTO, J. T., SHILONI, E., EISENTHAL, A., AND ROSENBERG, S. A.: Combined effects of chemotherapy and interleukin 2 in the therapy of mice with advanced pulmonary tumors. Cancer Res. **48:** 122–129, 1988.

PEARLSTEIN, K. T., PALLADINO, M. A., WELTE, K., AND VILCEK, J.: Purified human interleukin-2 enhances induction of immune interferon. Cell. Immunol. **80:** 1–9, 1983.

PFIZENMAIER, K., SCHEURICH, P., SCHLUTER, C., AND KRONKE, M.: Tumor necrosis factor enhances HLA-A, B, C and HLA-DR gene expression in human tumor cells. J. Immunol. **138:** 975–980, 1987.

PIERCE, E. C.: Renal lymphatics. Anat. Rec. **90:** 315–329, 1944.

RALPH, P., NAKOINZ, I., AND RENNICK, D.: Role of rIL-2, IL-4 and IFNs in stimulating macrophage antibody-dependent tumoricidal activity. J. Exp. Med. **167:** 712–717, 1988.

RANNIE, G. H., AND DONALD, K. J.: Estimation of the migration of thoracic duct lymphocytes to non-lymphoid tissues. Cell. Tissue Kinet. **10:** 523–541, 1977.

REDDEHASE, M. J., MUTTER, W., AND KOXAINOWSKI, U.: In vivo application of recombinant interleukin 2 in the immunotherapy of established cytomegalovirus infection. J. Exp. Med. **165:** 650–656, 1987.

REISBECK, K., ANDERSSON, J., GULLBERG, M., AND FORSGREN, A.: Fluorinated 4-quinolones induce hyperproduction of IL-2. Proc. Nat. Acad. Sci. USA **86:** 2809–2813, 1989.

RICCARDI, C., GIAMPIETRI, A., MIGLIORATI, G., CANNARILE, L., D'ADAMIO, L., AND HERBERMAN, R. B.: Generation of mouse natural killer (NK) cell activity: effect of interleukin-2 (rIL-2) and interferon (IFN) on the in vivo development of natural killer cells from bone marrow (BM) progenitor cells. Int. J. Cancer **38:** 553–562, 1986.

ROBINS, R. A., AND BALDWIN, R. W.: T-cell subsets in tumour rejection responses. Immunol. Today **6:** 55–58, 1985.

ROSENBERG, S. A., AND TERRY, W. D.: Passive immunotherapy of cancer in animals and man. Adv. Cancer Res. **25:** 323–388, 1977.

ROSENBERG, S. A., LOTZE, M. T., MUUL, L. M., CHANG, A. E., AVIS, F. P., LEITMAN, S., LINEHAN, W. M., ROBERTSON, C. N., LEE, R. E., RUBIN, J. T.,

SEIPP, C. A., SIMPSON, C. G., AND WHITE, D. E.: A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N. Engl. J. Med. **316:** 889–97, 1987.

ROSENBERG, S. A., LOTZE, M. T., MUUL, L. M., LEITMAN, S., CHANGE, A. E., ETTINGHAUSEN, S. E., MATORY, Y. L., SKIBBER, J. M., SHILONI, E., VETTO, J. T., SEIPP, C. A. SIMPSON, C., AND REICHERT, C. M.: Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N. Engl. J. Med. **313:** 1485–1492, 1985b.

ROSENBERG, S. A., MULE, J. J., SPIESS, P. J., REICHERT, C. M., AND SCHWARZ, S. L.: Regression of established pulmonary metastases and subcutaneous tumor mediated by the systemic administration of high-dose recombinant Interleukin-2. J. Exp. Med. **161:** 1169–1188, 1985a.

ROSENSTEIN, M., ETTINGHAUSEN, S. E., AND ROSENBERG, S. A.: Extravasation of intravascular fluid mediated by the systemic administration of recombinant interleukin 2. J. Immunol. **137:** 1735–1742, 1986.

ROSENSTEIN, M., YRON, I., KAUFMANN, Y., AND ROSENBERG, S. A.: Lymphokine activated killer cells: lysis of fresh syngeneic NK-resistant murine tumor cells by lymphocytes cultured in interleukin-2. Cancer Res. **44:** 1946–1953, 1984.

ROUSE, B. T., MILLER, L. S., TURTINEN, L., AND MOORE, R. N.: Augmentation of immunity to herpes simplex virus by in vivo administratin of interleukin 2. J. Immunol. **134:** 926–930, 1985.

RUBIN, L. A., JAY, G., AND NELSON, D. L.: The released interleukin-2 receptor binds interleukin-2 efficiently. J. Immunol. **137:** 3841–3844, 1986.

RUBIN, L. A., KURMAN, C. C., FRITZ, M. E., BIDDISON, W. E., BOUTIN, B., YARCHOAN, R., AND NELSON, D. L.: Soluble IL-2 receptors are released from activated human lymphoid cells in vitro. J. Immunol. **135:** 3172–3177, 1985.

SALTZMAN, E. M., THOM, R. R ., AND CASNELLIE, J. E.: Activation of a tyrosine protein kinase is an early event in the stimulation of T lymphocytes by interleukin-2. J. Biol. Chem. **263:** 6956–6959, 1988.

SALUP, R. R., AND WILTROUT, R. H.: Adjuvant immunotherapy of established murine renal cancer by interleukin 2-stimulated cytotoxic lymphocytes. Cancer Res. **46:** 3358–3363, 1986b.

SALUP, R. R., AND WILTROUT, R. H.: Treatment of adenocarcinoma in the peritoneum of mice: chemoimmunotherapy with rIL-2-stimulated cytotoxic lymphocytes as a model for treatment of minimal residual disease. Cancer Immunol. Immunother. **22:** 31–36, 1986a.

SALUP, R. R., HERBERMAN, R. B., CHIRIGOS, M. A., BACK, T., AND WILTROUT, R. H.: Therapy of peritoneal murine cancer with biological response modifiers. J. Immunopharmacol. **7:** 417–436, 1985.

SARIS, S. C., ROSENBERG, S. A., FRIEDMAN, R. B., RUBIN, J. T., BARBA, D., AND OLDFIELD, E. H.: Penetration of rIL-2 across the blood cerebrospinal fluid barrier. J. Neurosurg. **69:** 29–34, 1988.

SCHAUENSTEIN, K., KROMER, G., SUNDICK, R. S., AND WICK, G.: Enhanced response to Con A and production of TCGF by lymphocytes of obese strain (OS) chickens with spontaneous autoimmune thyroiditis. J. Immunol. **134:** 872–879, 1985.

SCHEURICH, P., THOMA, B., UCER, U., AND PFIZENMAIER, K.: Immunoregulatory activity of recombinant human tumor necrosis factor (TNF)-alpha: Induction of TNF receptors on human T cells and TNF-alpha-mediated enhancement of T cell responses. J. Immunol. **138:** 1786–1790, 1987.

SCHOEFL, G. L.: The migration of lymphocytes across the vascular endothelium in lymphoid tissue: a reexamination. J. Exp. Med. **136:** 568–588, 1972.

SCHWARTZ, A., SUTTON, S. L., AND GERSHON, R. K.: Regulation of in vitro cytotoxic T lymphocyte generation. II. Demonstration of noncytotoxic alloantigen-specific suppressor T lymphocytes. Eur. J. Immunol. **12:** 380–386, 1982.

SEMENZATO, G., BAMBARA, L. M., BIASI, D., FRIGO, A., VINANTE, F., ZUPPINI, B., TRENTIN, L., FERUGLIO, C., CHILOSI, M., AND PIZZOLO, G.: Increased serum levels of soluble interleukin-2 receptor in patients with systemic lupus erythematosus and rheumatoid arthritis. J. Clin. Immunol. **8:** 447–452, 1988.

SHALABY, M. R., SVEDERSKY, L. P., MCKAY, P. A., FINKLE, B. S., AND PALLADINO, M. A.: In vivo augmentation of NK activity by combined treatment with recombinant gamma interferon and rIL-2. J. Interferon Res. **5:** 571–581, 1985.

SHARMA, S. D., HOFFLIN, J. M., AND REMINGTON, J. S.: In vivo recombinant interleukin 2 administration enhances survival against a lethal challenge with Toxoplasma gondii. J. Immunol. **135:** 4160–4163, 1985.

SHERBURNE, C. G., CONDIE, R. M., NNADI, E. C., AND ADLIN, M.: The use of allogeneic stimulation and exogenous interleukin for syngeneic tumor rejection. Transplant. Proc. **19:** 628–631, 1987.

SHILONI, E., LAFRENIERE, R., MULE, J. J., SCHWARTZ, S. L., AND ROSENBERG, S. A.: Effect of immunotherapy with allogeneic lymphokine-activated killer cells and recombinant interleukin 2 on established pulmonary and hepatic metastases in mice. Cancer Res. **46:** 5633–5640, 1986.

SILAGI, S., AND SCHAEFER, A. E.: Successful immunotherapy of mouse melanoma and sarcoma with recombinant interleukin-2 and cyclophosphamide. J. Biol. Response Modif. **5:** 411–422, 1986.

SILAGI, S., DUTKOWSKI, R., AND SCHAEFER, A.: Eradication of mouse melanoma by combined treatment with recombinant human interleukin 2 and recombinant murine interferon-gamma. Int. J. Cancer **41:** 315–322, 1988.

SOBEL, A., AND LAGRUE, G.: Role of vascular permeability-increasing factor released by lymphocytes in renal pathology. *In* Lymphokine Reports, ed. by E. Pick, vol. I, pp. 211–230, Academic Press, New York, 1980.

SPIEGEL, R. J.: The alpha interferons: clinical overview. Semin. Oncol. **14** (suppl 2): 1–12, 1987.

SPRENT, J.: Fate of H_2-activated T lymphocytes in syngeneic hosts. I. Fate in lymphoid tissues and intestines traced with ^{3}H-thymidine, ^{125}I-deoxyuridine and ^{51}chromium. Cell. Immunol. **21:** 278–302, 1976.

STEEG, P. S., MOORE, R. N., JOHNSON, H. M., AND OPPENHEIM, J. J.: Regulation of murine macrophage Ia antigen expression by a lymphokine with immune interferon activity. J. Exp. Med. **156:** 1780–1793, 1982.

STELLER, E. P., EGGERMONT, A. M., MATTHEWS, W., AND SUGARBAKER, P. H.: Recruitment of inflammatory cells to a tumor deposit potentiates the immunotherapeutic effects of interleukin-2. Cancer Immunol. Immunother. **23:** 165–168, 1986.

STEVENS, S. K., WEISSMAN, I. L., AND BUTCHER, E. C.: Differences in the migration of B and T lymphocytes: organ-selective localization in vivo and the role of lymphocyte-endothelial cell recognition. J. Immunol. **128:** 844–851, 1982.

STROBER, W., AND WALDMANN, T. A.: The role of the kidney in the metabolism of plasma proteins. Nephron **13:** 35e46, 1974.

TAKEI, F., LEVY, J. G., AND KILBURN, D. G.: In vitro induction of cytotoxicity against syngeneic mastocytoma and its suppression by spleen and thymus cells from tumor-bearing mice. J. Immunol. **116:** 288–293, 1976.

TALMADGE, J. E.: Immunoregulation and immunostimulation of murine lymphocytes by human interleukin-2. J. Biol. Response Modif. **4:** 18–34, 1985.

TALMADGE, J. E.: Therapeutic potential of cytokines. Clin. Immunol. Newslett. **8:** 108–112, 1987.

TALMADGE, J. E., AND HERBERMAN, R. B.: The preclinical screening laboratory: evaluation of immunomodulatory and therapeutic properties of biological response modifiers. Cancer Treat. Rep. **70:** 171–182, 1986.

TALMADGE, J. E., HERBERMAN, R. B. CHIRIGOS, M. A., MALUISH, A. E., SCHNEIDER, M. A., ADAMS, J. S., PHILIPS, H., THURMAN, G. B., VARESIO, L., LONG, C., OLDHAM, R. K., AND WILTROUT, R. H.: Hyporesponsiveness to augmentation of murine natural killer cell activity in different anatomical compartments by multiple injections of various immunomodulators including recombinant intererons and interleukin 2. J. Immunol. **135:** 2483–2491, 1985.

TALMADGE, J. E., PHILLIPS, H. SCHINDLER, J., TRIBBLE, H., AND PENNINGTON, R.: A systematic preclinical study on the therapeutic properties of recombinant human interleukin 2 for the treatment of metastatic disease. Cancer Res. **47:** 5725–5732, 1987.

TANIGUCHI, T., MATSUI, H., FUJITA, T., TAKAOKA, C., KASHIMA, N., YOSHIMOTO, R., AND HAMURO, J.: Structure and expression of a cloned cDNA for human interleukin-2. Nature (Lond.) **302:** 305–310, 1983.

TARLETON, R. L., AND KUHN, R. E.: Restoration of in vitro immune responses of spleen cells from mice infected with *Trypanosoma cruzi* by supernatants containing interleukin 2. J. Immunol. **133:** 1570–1575, 1984.

TEMPELIS, C. H., HALA, K., KROMER, G., SCHAUENSTEIN, K., AND WICK, G.: Failure to alter neonatal transplantation tolerance by the injection of interleukin 2. Transplantation (Baltimore) **45:** 449–451, 1988.

TEZABWALA, B. U., JOHNSON, P. M., AND REES, R. C.: Inhibition of pregnancy viability in mice following IL-2 administration. Immunology **67:** 115–119, 1989.

THIJS, L. G., STRACK VAN SCHIJNDEL, R. J. M., AND WAGSTAFF, J.: Il-2 induces clinical features of septic shock. Circ. Shock **24:** 201–207, 1988.

THOMAN, M. L., AND WEIGLE, W. O.: Lymphokines and aging: interleukin 2 production and activity in aged animals. J. Immunol. **127:** 2102–2106, 1981.

THOMAN, M. L., AND WEIGLE, W. O.: Reconstitution of in vivo cell-mediated lympholysis responses in aged mice with interleukin 2. J. Immunol. **134:** 949–952, 1985.

THOMPSON, J. A., PEACE, D. J., KLARNET, J. P., KERN, D. E., GREENBERG, P. D., AND CHEEVER, M. A.: Eradication of disseminated murine leukemia by treatment with high-dose interleukin 2. J. Immunol. **137:** 3675–3680, 1986.

THURMAN, G. B., MALUISH, A. E., ROSSIO, J. L., SCHLICK, E., ONOZAKI, K., TALMADGE, J. E., PROCOPIO, A., ORTALDO, J. R., RUSCETTI, F. W., STEVENSON, H. C., CANNON, G. B., IYAR, S., AND HERBERMAN, R. B.: Comparative evaluation of multiple lymphoid and recombinant interleukin-2 preparations. J. Biol. Response Modif. **5:** 85–107, 1986.

TING, C-C., YANG, S. S., AND HARGROVE, M. E.: Induction of suppressor T cells by interleukin 2. J. Immunol. **133:** 261–266, 1984.

TRACEY, K. J., BEUTLER, B., LOWRY, S. F., MERRYWEATHER, J., WOLPE, S., MILSARK, I. W., HARIRI, R. J., FAHEY, T. J., ZENTELLA, A., ALBERT, J. D., SHIRES, G. T., AND CERAMI, A.: Shock and tissue injury induced by recombinant human cachectin. Science (Wash. DC) **234:** 470–474, 1986.

TURCOTTE, R.: Evidence for two distinct populations of suppressor cells in the spleens of *Mycobacterium bovis* BCG-sensitized mice. Infect. Immun. **34:** 315–322, 1981.

TUTTLE, R. S., AND BOPPANA, D. P.: Antihypertensive effect of interkeukin-2. Hypertension **15:** 89–94, 1990.

URBAN, J. L., BURTON, R. C., HOLLAND, J. M., KRIPKE, M. L., AND SCHREIBER, H.: Mechanisms of syngeneic tumor rejection: susceptibility of host-selected progressor variants to various immunological effector cells. J. Exp. Med. **155:** 557–573, 1982.

VAAGE, J., PAULY, J. L., AND HARLOS, J. P.: Influence of the administration schedule on the therapeutic effect of interleukin-2. Int. J. Cancer **39:** 530–533, 1987.

VALGE, V. E., WONG, J. G., DATLOF, B. M., SINSKEY, A. J., AND RAO, A.: Protein kinase C is required for responses to T cell receptor ligands but not to interleukin-2 in T cells. Cell **55:** 101–112, 1988.

VOSE, B. M.: Quantitation of proliferative and cytotoxic precursor cells directed

against human tumours: limiting dilution analysis in peripheral blood and at the tumour site. Int. J. Cancer. **30:** 135–142, 1982.

WAHL, S. M., AND WAHL, L. M.: The modulation of fibroblast growth and function by monokines and lymphokines. *In* Lymphokines, ed. by M. E. Pick, vol. 2, pp. 197–201, Academic Press, New York, 1980.

WALDMAN, T. A., GOLDMAN, C. K., ROBB, R. J., DEPPER, J. M., LEONARD, W. J., SHARROW, S. O., BONGIOVANNI, K. F., KORSMEYER, S. J., AND GREENE, W. C.: Expression of interleukin 2 receptors on activated human B cells. J. Exp. Med. **160:** 1450–1466, 1984.

WALDMANN, T. A.: The Structure, function and expression of IL-2 receptors on normal and malignant lymphocytes. Science (Wash. DC) **232:** 727–732, 1986.

WALDMANN, T. A.: The multichain IL-2 Receptor: from the gene to the bedside. Harvey Lect. **82:** 1–17, 1988.

WAN, Y-J. Y., ORRISON, B. M., LIEBERMAN, R., LAZAROVICI, P., AND OZATO, K.: Induction of major histocompatibility class I antigens by interferons in undifferentiated F9 cells. J. Cell. Physiol. **130:** 276–283, 1987.

WANG, A., LU, S-D., AND MARK, D. F.: Site-specific mutagenesis of the human interleukin-2 gene: structure-function analysis of the cysteine residues. Science (Wash. DC) **224:** 1431–1433, 1984.

WEBER, J. S., JAY, G., TANAKA, K., AND ROSENBERG, S. A.: Immunotherapy of a murine tumor with interleukin-2: increased sensitivity after MHC class I gene transfection. J. Exp. Med. **166:** 1716–1733, 1988.

WEINBERG, A., RASMUSSEN, L., AND MERIGAN, T. C.: Acute genital infection in guinea pigs: effect of recombinant interleukin-2 on Herpes simplex virus type 2. J. Infect. Dis. **154:** 134–140, 1986.

WEISBERGER, A. S., GUYTON, R. A., HEINLE, R. W., AND STORAASLI, J. P.: The role of the lungs in the removal of transfused lymphocytes. Blood **6:** 916–925, 1952.

WEST, W. H., TAUER, K. W., YARNELLI, J. R., MARSHALL, G. L., ORR, D. W., THURMAN, G. B., AND OLDHAM, R. K.: Constant infusion recombinant interleukin-2 in adoptive immunotherapy of advanced cancer. *In* Biological Therapy of Cancer, A 1986 Update., Williams & Wilkins, Baltimore, 1986.

WEYAND, C. M., GORONZY, J., DALLMAN, M. J., AND FATHMAN, C. G.: Administration of recombinant interleukin 2 in vivo induces a polyclonal IgM response. J. Exp. Med. **163:** 1607–1612, 1986.

WILLIAMS, D. P., PARKER, K., BACHA, P., BISHAI, W., BOROWSKI, M., GENBAUFFE, F., STROM, T. B., AND MURPHY, J. R.: Prevention of allograft rejection by engineered conjugates of interleukin-2 and dephtheria toxin. Protein Engineering **1:** 493–498, 1987.

WILLOUGHBY, D. A.: Mediation of increased vascular permeability in inflammation. *In* The inflammatory process, ed. by B. W. Zweifach, L. Grant and R. T. McCluskey, vol. II, 2nd ed., pp. 303–331, Academic Press, New York, 1973.

WINKELHAKE, J. L., ELCOMBE, B. M., AND HODACH, A.: Immunological block to synthetic alpha-melanocyte-stimulating hormone: melanocyte interaction by antibodies isolated from cell-column immunoadsorbents. Cancer Res. **39:** 3058–3064, 1979.

WINKELHAKE, J. L., STAMPFL, S., AND ZIMMERMAN, R. J.: Synergistic effects of combination therapy with human recombinant interleukin-2 and tumor necrosis factor in murine tumor models. Cancer Res. **47:** 3948–3953, 1987.

WOCHNER, R. D., STROBER, W., AND WALDMANN, T. A.: The role of the kidney in the catabolism of Bence Jones proteins and immunoglobulin fragments. J. Exp. Med. **126:** 207–221, 1967.

XUE, B., DERSARKISSIAN, R. M., BAER, R. L., THORBECKE, G. J., AND BELSITO, D. V.: Reversal by lymphokines of the effect of cyclosprin A on contact sensitivity and antibody production in mice. J. Immunol. **136:** 4128–4133, 1986.

YE, Q-W., AND MOKYR, M. B.: Cyclophosphamide-induced appearance of immunopotentiating T-cells in the spleens of mice bearing a large MOPC-315 tumor. Cancer Res. **44:** 3873–3879, 1984a.

YE, Q-W., MOKYR, M. B., PYLE, J. M., AND DRAY, S.: Suppression of antitumor immunity by macrophages in spleens of mice bearing a large MOPC-315 tumor. Cancer Immunol. Immunother. **16:** 162–169, 1984b.

YOUNG, M. R., AND KNIES, S.: Prostaglandin E production by Lewis lung carcinoma: mechanism for tumor establishment in vivo. J. Natl. Cancer Inst. **72:** 919–922, 1984.

YRON, I., WOOD, T. A., SPIESS, P., AND ROSENBERG, S. A.: In vitro growth of murine T cells. V. The isolation and growth of lymphoid cells infiltrating syngeneic solid tumors. J. Immunol. **125:** 238–245, 1980.

ZEIDMAN, I.: Metastasis: a review of recent advances. Cancer Res. **17:** 157–162, 1967.

ZIMMERMAN, R. J., AUKERMAN, S. L., KATRE, N. V., WINKELHAKE, J. L., AND YOUNG, J. D.: Schedule dependency of the antitumor activity and toxicity of polyethylene glycol-modified interleukin 2 in murine tumor models. Cancer Res. **49:** 6521–6528, 1989a.

ZIMMERMAN, R. J., GAUNY, S., CHAN, A., LANDRE, P., AND WINKELHAKE, J. L.: Sequence dependence of murine tumor therapy with human recombinant tumor necrosis factor and interleukin-2. J. Natl. Cancer Inst. **81:** 227–231, 1989b.