Reprint Series
27 May 1988, Volume 240, pp. 1169–1176

SCIENCE

Interleukin-2: Inception, Impact, and Implications

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Interleukin-2 (IL-2), the first of a series of lymphocytotrophic hormones to be recognized and completely characterized, is pivotal for the generation and regulation of the immune response. A T lymphocyte product, IL-2 also stimulates T cells to undergo cell cycle progression via a finite number of interactions with its specific membrane receptors. Because T cell clonal proliferation after antigen challenge is obligatory for immune responsiveness and immune memory, the IL-2–T cell system has opened the way to a molecular understanding of phenomena that are fundamental to biology, immunology, and medicine.

THE DISCOVERY OF INTERLEUKIN-2 (IL-2) (*1*) AND THE investigations that established it as the lymphocytotrophic hormone responsible for signaling T lymphocyte (T cell) proliferation (*2–10*) have had an extraordinary impact on the discipline of immunology, transforming the very nature of immunologic inquiry. IL-2 has become the prototype lymphokine, bringing to immunology concepts long familiar to investigators schooled in the principles of endocrinology, pharmacology, and enzymology. In particular, the affinity of ligand-receptor interactions, the structure-function relations of lymphokines and their receptors, receptor-mediated signal transduction mechanisms, and lymphokine induction of specific gene expression have now become central issues in immunology research. This reflects a historic shift from the cellular immunology of the 1960s and 1970s, which had focused on antigens as solely responsible for the intracellular biochemical reactions that promote cellular proliferation and differentiation; indeed, actual contact among T cells, B cells, and macrophages had been considered an essential prerequisite for effecting intercellular communication. Consequently, antigen-receptor idiotypic networks, cell-cell interactions, suppressor T cells, and antigen-specific helper and suppressor factors were believed to account for regulation of the immune response. Now, intercellular communication is firmly established as being driven by lymphokine molecules.

Lymphokine research emerged from rather obscure origins to become a legitimate endeavor as a result of advances made just within the past decade. During this rather brief span it has become evident that the immune system uses hormonal mechanisms to direct virtually all aspects of the response to antigen, including proliferative clonal expansion and differentiation to specialized functions such as T cell cytolysis, T cell help, and even antibody production by B cells. The fact is that lymphokines serve to transfer control of the immune response from external environmental antigens, which initiate the process, to an internal regulatory system consisting of specific ligands and receptors, which actually stimulate distinct intracellular pathways that carry forward the characteristic cellular changes. Accordingly, the vocabulary of immunologists and the kinds of experiments they perform are no longer foreign to those from other biologic disciplines. Instead, experiments with lymphocytes and lymphokines have become the cells and molecules of choice for an array of physiologic, biologic, biochemical, and genetic studies directed toward comprehending just how cells perform in response to separate and distinguishable external stimuli.

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Mitogenic Lymphokines and T Cell Clones

The beginning of understanding T cell growth can be traced to 1960, when Nowell (*11*) discovered that plant lectins such as phytohemagglutinin (PHA) are mitogenic for lymphocytes. Before this finding, lymphocytes were considered to be terminally differentiated end-stage cells, quite incapable of further proliferation. However, as a result of Nowell's observation still other mitogenic lectins were sought and rapidly identified, and soon thereafter specific antigens were shown also to trigger a proliferative response in vitro. As a consequence, throughout the next 20 years, antigen-specific T cell proliferation became virtually synonymous with cell-mediated immunity.

In 1965, two simultaneous reports (*12*) contained descriptions of the first soluble mitogenic factors derived from medium conditioned by allogeneic mixed leukocyte cultures. These supernatants surely contained the molecule we now recognize as IL-2. However, at the time, most immunologists were rather indifferent to mitogenic lymphokines, having assumed that even if they existed, they were less important than specific antigen. Thus, mitogenic lymphokines were thought to play a nutritional or supportive role, merely amplifying a process signaled entirely by antigen. No one thought it possible that the lymphokines themselves could be responsible for stimulating cell division.

Initially it was assumed that lymphocytes were the only source of all the mitogenic factors found in activated leukocyte cultures, as evidenced by adoption of the term lymphokine, which was coined to refer to the mediators of cellular immunity (*13*). However, as far back as 1970, Bach *et al.* (*14*) reported that adherent nonlymphoid accessory cells released substances with mitogenic activity into culture media, and, shortly thereafter, in 1972 Gery *et al.* (*15*) proposed the term lymphocyte-activating factor (LAF) to distinguish macrophage-derived activities from those that might have originated in lymphocytes.

During the next several years, investigators were largely concerned with attempts to concentrate and purify the molecule or molecules responsible for the mitogenic activities. One popular notion at the time was that whole families of molecules were

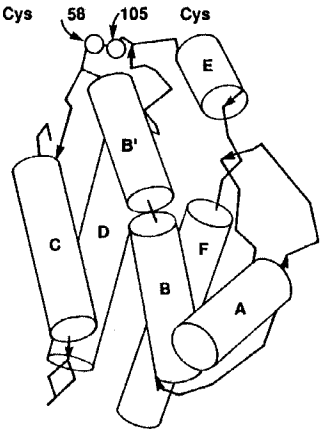

Fig. 1. Schematic drawing of IL-2 based on data obtained by x-ray crystallography. The alpha helices are represented as cylinders and are lettered sequentially from the amino terminus. [Redrawn from B. J. Brandhuber, T. Boone, W. C. Kenney, and D. B. McKay (*34*), with permission]

responsible for each activity measured; isolation of individual molecules was envisioned by some as an essentially insurmountable task. Moreover, even if it was assumed that the activities resulted from the interaction of a single lymphokine molecule with an individual target cell, this would be difficult to prove. At that phase of the research, lymphokine activities were generally assayed by using heterogeneous target cell populations, usually mixtures of T cells, B cells, and macrophages, so that it was virtually impossible to ascertain whether the activity being measured resulted from a direct or indirect interaction with the presumed target cell. To complicate the task even further, the only biochemical separative procedures then available were laborious and mostly yielded poor resolution. All of this compounded the problem, particularly when it was finally realized that exceedingly low lymphokine concentrations were responsible for the measurable activities. In this early work, many liters of conditioned media would have been necessary to achieve even the simplest degree of purification.

In 1976 a report appeared that eventually led to the development of new approaches for overcoming these experimental problems. Morgan *et al.* (*16*) showed that normal human T cells could be cultured for extended periods, even up to a year, in media that had been "conditioned" by growth of PHA-stimulated human peripheral mononuclear cells. This report went unnoticed by most immunologists because it dealt with a way to culture T cells from bone marrow, a site not ordinarily viewed as a source of mature T cells. Moreover, even though the cells continued to proliferate in conditioned medium, they appeared to be immunologically immature, as they could not be shown to perform any antigen-specific functions. Consequently, the phenomenon was dismissed as merely another aspect of the antigen-nonspecific nature of mitogenic factors in conditioned media; indeed this alone was more than enough reason to assure that it would be ignored by the immunology establishment.

During this period, experiments at Dartmouth were going on to generate cytolytic T cells specific for murine tumor antigens. Although tumor-specific cytolytic T cells could be detected, they could be maintained in proliferative culture for only a few days (*17*). Accordingly, after the report by Morgan *et al.* (*16*), conditioned medium was added to the cultures to try to retain the proliferation of antigen-specific cells that had been selected for by repetitive in vivo and in vitro immunizations. In view of the prevailing immunologic dogma that only antigen itself could initiate and sustain T cell proliferation, it seemed at the time that this type of experiment was destined to fail. Moreover, it was considered highly unlikely that functionally differentiated cells could be made to proliferate indefinitely. However, remarkably enough, murine cytolytic T lymphocyte lines (CTLL) specific for tumor antigens were established with ease (*18*). Indeed, even now, 10 years later, the actual original CTLL are still in continuous culture.

The key to the successful long-term growth of antigen-specific T cells was the realization that antigen was essential only to render the cells responsive to the mitogenic factors present in conditioned medium. Contrary to the prevailing belief, findings with the antigen-specific CTLL led to the compelling conclusion that the primary mitogenic effect had to be attributable to something separate and apart from antigen. This understanding prompted studies on the generation of human antigen-specific cytolytic T cells (*19*), which established the biologic generality of the phenomenon. Also, experiments to clone the CTLL demonstrated that clones could be generated easily by limiting dilution. Even without feeder cells, but via conditioned medium, almost 100% plating efficiency was obtained, and of the clones isolated, 80% proved to be cytolytic (*20*).

The ability to generate and maintain antigen-specific cloned T cells made feasible studies that had previously been impossible. Consequently, antigen-specific T cell clones have had an impact on immunology comparable to that of monoclonal immunoglobulin–secreting plasmacytomas. For example, the use of antigen-specific cloned T cells made possible studies on the fine specificity of major histocompatibility complex (MHC)–restricted responses, for which only ambiguous data could be obtained when heterogeneous T cell populations were used (*21*). Furthermore, the ability to generate large numbers of cloned functional T cells permitted definitive biochemical and molecular studies that previously had been quite unapproachable. In particular, cloned T cells were instrumental for studies that finally identified the T cell antigen receptor as a 90-kD disulfide-linked heterodimer (*22*). The availability of cytolytic T cell clones also made feasible studies on the molecular mechanisms of T cell–mediated cytolysis; one of the cell lines used to isolate and characterize cytolytic granules was actually CTLL-2, one of the original IL-2–dependent cell lines (*23*). Thus, cloned antigen-specific T cells really proved to be the unique cellular reagents predicted (*18–20*).

The First Interleukins

For studies on the nature of the mitogenic lymphokines in conditioned media, the development of cloned T cell populations was the breakthrough needed to interpret the results of our experiments unambiguously. Since cloned CTLL are the asexual progeny of a single cell, the problem of target cell heterogeneity was finally resolved, thereby allowing us to concentrate on reducing the complexity of the activities in conditioned medium by defining the biologic and biochemical characteristics of the active mitogenic molecule or molecules. Having cloned CTLL, we fashioned a rapid and sensitive bioassay to monitor the mitogenic activity that sustained long-term T cell growth (*1*). This assay is performed in such a way that it is possible to quantitate the concentration of the activity in various experimental samples by titration and probit analysis, procedures adapted from interferon assays that had been used previously in the laboratory for measuring erythropoietin (*24*).

Biologic experiments established T cells as the source of the substance with mitogenic activity (*1–3*), thereby identifying it as a lymphokine. Also, since T cells respond to this activity, the term T cell growth factor (TCGF) was coined (*1–3*) to describe and distinguish the activity from any mitogenic factors elaborated by macrophages. In this regard, a distinction between LAF and TCGF was possible only because LAF possessed no growth-promoting activity for the CTLL (*3*). Even so, LAF clearly stimulated the proliferation of thymocytes and macrophage-depleted T cells, as did TCGF. Accordingly, to reconcile this seeming paradox, a series of

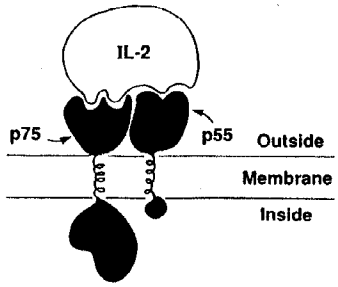

Fig. 2. The bimolecular structure of high-affinity IL-2 receptors. The area of contact between IL-2 and the two IL-2–binding proteins is depicted as quite large to account for experimental results showing that the folded tertiary structure of IL-2 is required for high-affinity binding and that the binding sites on the two chains of the receptor are independent but cooperative (*43–47*). The two chains are not connected by covalent disulfide bonds and their cooperative binding of IL-2 is easily disrupted by monoclonal antibodies reactive with p55 β chain (*46*). Since the p75 α chain contains the structures responsible for signaling internalization and cell growth (*46, 55, 56*), its cytoplasmic domain is depicted as being larger than the rather small cytoplasmic domain of the p55 β chain, which contains only 13 residues (*39*).

experiments finally revealed that LAF functioned to potentiate the production of TCGF (*3–6*), thereby providing a convincing explanation for the well-documented, but poorly understood, accessory cell requirement for optimal T cell proliferation. Moreover, these observations served as both the rationale and impetus for adoption of the term interleukin (between leukocytes) and for LAF to be identified as IL-1 and for TCGF to be designated IL-2 (*25*).

Although the interleukin nomenclature was intended to clarify and simplify the mitogenic lymphokine terminology, the change in names was really premature in that the activities had not yet been ascribed to separate molecules. For example, the LAF activity found in macrophage-conditioned media has never been convincingly ascribed to the molecules now designated IL-1α and IL-1β. However, another macrophage-derived cytokine (provisionally designated IL-6) has recently been shown to facilitate IL-2 production by purified T cells, as had LAF activity in unfractionated macrophage-conditioned media (*26*). Consequently, the exact function of the molecules designated IL-1α and IL-1β are now being questioned, and many of the LAF activities described originally may actually be mediated by IL-6.

Interleukin-2

Because the IL-2 bioassay is rapid and quantitative, experiments to purify and analyze the molecular characteristics of TCGF proceeded rapidly. Biochemical analyses revealed that all of the activity could be ascribed to a glycoprotein with a single molecular size of 15.5 kD and a single isoelectric point (*p*I 8.2) after removal of charged sialic acid residues (*7*). Thus, the apparent molecular heterogeneity detected in earlier experiments (*27*) was explained by variable glycosylation and sialylation of a single protein, and these studies served as the first accurate biochemical description of IL-2 (*7*). However, definitive evidence that IL-2 activity is attributable to a single molecule came only after the development of IL-2–reactive monoclonal antibodies, which provided a rapid one-step immunoaffinity purification of enough IL-2 to enable NH_2-terminal sequence determination (*10*).

The IL-2 bioassay and the description of the molecular characteristics of IL-2 were used by Taniguchi *et al.* (*28*) to isolate cDNA clones encoding IL-2 activity that predicted a mature protein of 15,420 daltons with an NH_2-terminal sequence identical to that found from analysis of the purified protein. Therefore, in only 5 years from the time of the development of the bioassay and the first experiments describing the biologic characteristics of TCGF (*1–3*), its molecular properties had been defined (*7*), monoclonal antibodies reactive with it were developed (*10*), it had been purified to homogeneity (*10*), and cDNA (*28*) as well as genomic DNA clones (*29*) had been isolated and sequenced. Such rapid progress was feasible largely because of the specificity and sensitivity of IL-2 detection afforded by the IL-2 bioassay.

The overall genomic organization of IL-2, which is encoded by four exons separated by one short and two long introns (*29*), is remarkably similar to the genomic organization of other cytokines, including IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (*30*). Consequently, it appears that a family of similar molecules could have evolved from a common genetic progenitor. In addition, such genomic structural homology suggests that each exon may well encode distinct elements (*31*) that are common to a single family of lymphokines. Thus, we anticipate structural similarities and functional cross-reactivity among individual lymphokines, even without obvious primary amino acid sequence homology.

With the availability of unlimited quantities of cloned IL-2–responsive T cells, the IL-2 bioassay, homogeneous recombinant IL-2, and an understanding of the novel characteristics of the IL-2 receptor, IL-2 has become an ideal immunoreactive molecule for studies of structure-activity relations. Thus far, we and others have shown that truncated segments of the IL-2 sequence cannot promote T cell growth (*32*), and the reduction of the disulfide bond between residues 58 and 105 destroys biologic activity (*33*), thereby indicating that the tertiary, folded structure of IL-2 is obligatory for stimulating growth. Recently, the crystal structure of IL-2 has been solved to 3.0 angstroms resolution (*34*), revealing that the core structure is made up of anti-parallel alpha helices and contains no segments of beta secondary structure (Fig. 1). These findings are consistent with our data from circular dichroism (*35*), and although only the spatial arrangement of the peptide backbone has been resolved thus far, the progress that has already been made indicates that the total crystallographic structure with the placement of side chains will be known soon. As the atomic coordinates become available, it will be possible to use the IL-2 structure to formulate models of the other lymphokines that appear to be members of this molecular family. Moreover, for structure-activity studies, the exact surface topography of the molecule will be particularly pertinent for experiments to identify the receptor-binding residues of IL-2.

Table 1. Kinetic and equilibrium IL-2 binding constants. Sources of chains are: α chain, YT-2C2 membranes; β chain, MT-1 cells; and α,β chains, induced YT membranes. Equilibrium constants are means (± SEM) from eight separate equilibrium binding experiments. [Reproduced by permission from H.-M. Wang and K. A. Smith (*46*)]

Chain	Kinetic binding constants			Equilibrium dissociation constants (*M*)	
	Dissociation (*k'*)		Association (*k*)	Kinetic (*k'/k*)	Equilibrium
	s^{-1}	$t_{1/2}$	$M^{-1}\,s^{-1}$		
p75 α	2.5×10^{-4}	46 minutes	3.8×10^{5}	0.7×10^{-9}	$1.2\ (\pm 0.1) \times 10^{-9}$
p55 β	4.0×10^{-1}	1.7 seconds	1.4×10^{7}	2.9×10^{-8}	$1.4\ (\pm 0.1) \times 10^{-8}$
α,β	2.3×10^{-4}	50 minutes	3.1×10^{7}	0.7×10^{-11}	$1.3\ (\pm 0.1) \times 10^{-11}$

The Interleukin-2 Receptor

Although it was evident that T cells both produce and respond to IL-2, the mechanism whereby the molecule interacts with the cells to promote its effects remained unknown. However, our earliest experiments (*1*) pointed the way to a hormone-receptor interaction as most likely. IL-2 is consumed by activated T cells (*1*), and absorption experiments revealed that removal of the activity is dependent on time, temperature, and cell density (*36*). Therefore, it seemed a reasonable assumption that activated T cells express IL-2–specific receptors and that the disappearance of IL-2 activity would be attributable to receptor-mediated endocytosis with subsequent intracellular degradation.

Since hormone receptors are generally defined by use of radiolabeled ligand binding assays, IL-2 was radiolabeled biosynthetically, the product was purified, and binding experiments were performed. The first results were decisive and demonstrated that the characteristics of the binding sites met all of the criteria necessary for designating them as components of true hormone receptor molecules, including high affinity, saturability, ligand specificity, and target cell specificity (*8*). Of greater significance was the finding that the IL-2 concentrations that bind with high affinity to IL-2 receptors are the same as the concentrations of IL-2 that promote T cell proliferation in vitro; that is, the dissociation constant (K_d) is the same as the median effective concentration (EC_{50}), having a value of $10^{-11}M$ (*8*).

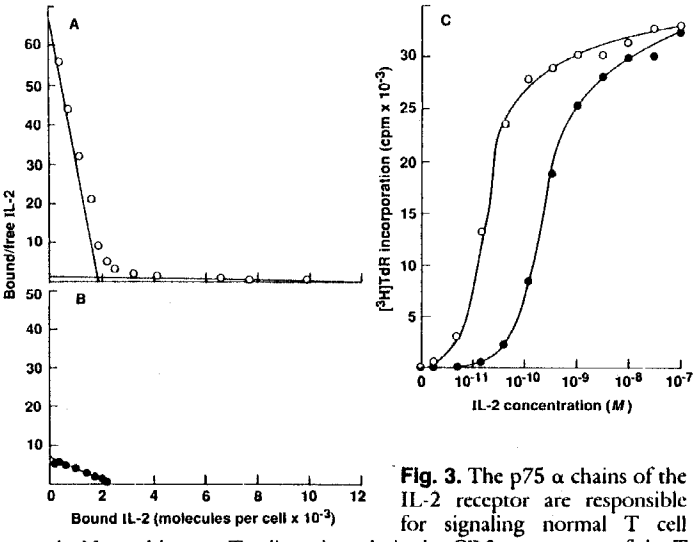

Fig. 3. The p75 α chains of the IL-2 receptor are responsible for signaling normal T cell growth. Normal human T cells activated via the CD3 component of the T cell antigen receptor were assayed for IL-2 receptor expression by a radioiodinated IL-2 binding assay (**A**) in the absence and (**B**) in the presence of a monoclonal antibody reactive with the IL-2 receptor p55 β chain. As shown in (A), there are two classes of IL-2 binding sites as indicated by Scatchard plot analysis of the binding data. Since the slopes of the data points yield the equilibrium binding constants and the *x*-axis intercepts give the receptor density, it is clear that there are ~2000 high-affinity ($K_d \simeq 3 \times 10^{-11}M$) binding sites per cell and ~11,000 low-affinity ($K_d \simeq 1 \times 10^{-8}M$) binding sites per cell. By comparison, in the presence of a saturating concentration of a monoclonal antibody to the p55 β chains (B), neither high-affinity nor low-affinity binding sites are detectable. Instead, the number of intermediate-affinity ($K_d \simeq 4 \times 10^{-10}M$) binding sites that appear is ~2000, indistinguishable from the former number of high-affinity receptors [compare (B) with (A)]. Since the monoclonal antibodies prevent high-affinity IL-2 binding and uncover isolated intermediate-affinity binding sites, the possibility that the cell growth signal requires high-affinity IL-2 binding could be tested. (**C**) The presence of the monoclonal antibody (*37*) does not prevent an IL-2–dependent growth response of normal T cells. Rather, the concentrations of IL-2 required to stimulate growth are increased and correspond to those necessary to bind to isolated p75 α chains ($EC_{50} \simeq 5 \times 10^{-10}M$ versus $K_d \simeq 4 \times 10^{-10}M$). [From H.-M. Wang and K. A. Smith (*46*), with permission]

Although the IL-2 binding characteristics of the IL-2 receptor were elucidated easily, the structural nature of the receptor proved elusive. Uchiyama *et al.* (*37*) developed monoclonal antibodies that were subsequently found to compete for radiolabeled IL-2 binding (*9*). However, these antibodies reacted with many more cell-surface molecules than could be detected by high-affinity radiolabeled IL-2 binding (*9*). Subsequent experiments showed that the large majority of the excess molecules reactive with the monoclonal antibodies also bind IL-2, but with a much lower affinity than the high-affinity IL-2 receptors (*38*). Isolation of cDNA encoding the putative IL-2 receptor as defined by the monoclonal antibody (*39*) did not solve the mystery of the nature of authentic high-affinity IL-2 receptors; transfection experiments with non-T cells revealed that this cDNA encodes a product that is only capable of binding IL-2 with low affinity (*40*). When further cDNA experiments indicated that high-affinity IL-2 receptors might be expressed by transfected T cells (*41*), it was postulated that a "converter" protein from T cells somehow imparted high affinity to the low-affinity binding protein (*42*). However, no one considered it likely that a separate IL-2 binding site might account for the data (*42*).

Within the past year, authentic, high-affinity IL-2 receptors have been found to consist of two distinct polypeptide chains, each of which contains an IL-2 binding site (*43*, *44*). In conformity with the convention for nomenclature established for other cell-surface receptors, the larger IL-2–binding protein (75 kD) is designated as the α chain, whereas the smaller protein (55 kD) is termed the β chain (*44*). Experiments detailing the distinct IL-2 binding characteristics of the two separate chains became possible only after the identification and isolation of leukemic cell clones that express solely p75 α chains (*43*, *44*). Moreover, the availability of these cell lines permitted the initial findings to be repeated and confirmed (*45*), establishing unanimity where previously only controversy had existed.

It is especially noteworthy that the p75 α chain and the p55 β chain are not connected via a covalent disulfide bond. Instead, these two chains apparently interact via noncovalent forces to form a heterodimeric high-affinity IL-2 receptor. Also, the p75 α chain IL-2 binding site is separate and distinct from the IL-2 binding site on the p55 β chain as depicted in Fig. 2. Evidence favoring this interpretation comes from a number of observations. For example, when expressed individually, the two chains bind IL-2 with distinct affinities; the p75 α chain binds IL-2 with a K_d of $10^{-9}M$, whereas the p55 β chain binds IL-2 with a K_d of $10^{-8}M$ (*44*). Furthermore, only when both chains are expressed together by the same cell are high-affinity IL-2 receptors (K_d, $10^{-11}M$) detectable (*44*). These findings essentially preclude the recognition of the same residues on IL-2 by the binding sites on both chains, a result that would lead to competition for IL-2 binding. Instead, cooperation between the binding sites on the two chains leads to the marked increase (100- to 1000-fold) in binding affinity of the α,β heterodimer. This model also provides a structural explanation for the requirement of the entire intact IL-2 molecule for high-affinity binding, since different residues of IL-2 bind to each chain.

A dynamic view of the structure-function relations of the two separate IL-2–binding proteins and how they cooperate to form high-affinity IL-2 receptors derives from kinetic binding studies (*46*, *47*). When expressed individually, each chain reacts with IL-2 very differently, as displayed by the kinetic and equilibrium binding constants shown in Table 1. On the basis of these results, we now recognize that IL-2 binds to and dissociates from p75 α chains relatively slowly, whereas it reacts very rapidly with p55 β chains. Even more intriguing are the kinetic binding data obtained when high-affinity receptors are analyzed (*46*); the association rate of the

heterodimer is contributed by the fast-reacting p55 β chain, whereas the dissociation rate is derived from the slow-reacting p75 α chain. Since the affinity of binding at equilibrium is determined by the ratio of the dissociation rate constant ($k' \sim 10^{-4}$ s^{-1}) and the association rate constant ($k \sim 10^7 M^{-1}$ s^{-1}), this unique kinetic cooperation between two lower affinity ligand binding sites results in a very high affinity receptor ($K_d = k'/k \simeq 10^{-11} M$). Thus far, the high-affinity IL-2 receptor is the only known example of this kind of receptor formed by making use of distinct binding sites on two separate chains. However, this structure may well reflect a general model for receptors that has been selected for and conserved, especially as it results in such a profound enhancement in the efficiency of the ligand-receptor interaction (*46*).

It is noteworthy that one of the leukemic cell lines found to express only p75 α chains is the gibbon ape cell line MLA-144 (*43*), inasmuch as this cell line proliferates in an autocrine fashion in response to the IL-2 that it produces itself (*48*). To determine whether p75 α chains also transduce the growth signal to normal T cells without the assistance of the p55 β chain, cells capable of binding IL-2 solely via the p75 α chain were created by using monoclonal antibodies (*9*) (Fig. 3). The antibody blocks IL-2 binding to p55 β chains and disrupts the subtle kinetic cooperation between the p75 α chain and the p55 β chain, so that high-affinity IL-2 binding disappears and is replaced by intermediate-affinity binding characteristic of isolated p75 α chains (compare Fig. 3A with Fig. 3B). In the presence of the antibody, IL-2 promotes the proliferation of normal human T cells at concentrations explicable only by interaction with p75 α chains alone (*46*) (Fig. 3C). Accordingly, each of the two IL-2–binding proteins contributes its unique kinetic binding characteristics to form the authentic high-affinity IL-2 receptor, but only p75 α chains stimulate T cell proliferation.

Since the p75 α chain was recognized only recently, its distribution on cells of lineages other than mature, activated T cells is still conjectural. However, p75 α chain expression has been reported on cells with natural killer activity (*49*), and this chain may also be operative in stimulating the proliferation of immature thymocytes. The expression of IL-2 receptors by B cells also needs further clarification. About tenfold fewer IL-2 receptors are expressed by activated murine and human B cells than by T cells, as quantitated with either radiolabeled IL-2 or monoclonal antibodies reactive with the p55 β chain (*50*).

Regulation of the T Cell Immune Response

Most circulating lymphocytes are quiescent; only upon introduction of antigen do specific clones of cells proliferate and differentiate into effector cells capable of mediating T cell cytolysis, T cell help, or antibody production. Moreover, upon clearance of the offending antigen, proliferation declines gradually and the cells eventually lose their capacity to perform their specialized effector functions. The immune system thus responds to changes in the internal molecular milieu that occur when foreign microbes and molecules are introduced, and it returns to its quiescent state when homeostasis has been achieved. The understanding that lymphokines such as IL-2 regulate this response now makes it possible to determine just how the system actually operates.

As evidenced by the results of the very first IL-2 experiments (*1*), resting T cells do not produce IL-2, nor are they capable of responding to IL-2 when it is added exogenously. It follows that signals emanating from the T cell antigen-receptor complex coordinate the transcriptional activation of both the IL-2 gene and the genes encoding IL-2 receptors (*51*). The concentration of IL-2

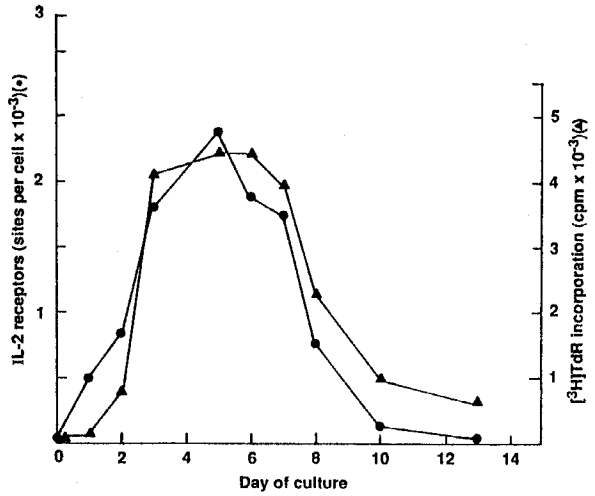

Fig. 4. The kinetics of IL-2 receptor expression and T cell proliferation in response to polyclonal stimulation. Most (≥98%) freshly isolated human peripheral mononuclear cells have no receptors for IL-2 and do not respond to IL-2 added exogenously. However, when stimulated with phytohemagglutinin, which activates virtually all T cells, individual cells gain IL-2 receptors (●) asynchronously, and 3 or 4 days are required before maximal levels of receptors are measurable. Proliferation, as monitored by tritiated thymidine ([^{3}H]TdR] incorporation (▲), parallels IL-2 receptor expression, and both decline to very low levels after 2 weeks of culture even when IL-2 is supplied exogenously. Consequently, the cell population reaccumulates in the resting phase of the cell cycle. [Redrawn from D. A. Cantrell and K. A. Smith (*52*), with permission]

produced and the period during which it is available then become primary determinants of the magnitude and extent of IL-2–promoted T cell proliferation. Expression of functional IL-2 receptors is the only other variable that ultimately determines just how long clonal expansion actually occurs after antigen activation. From studies performed in vitro, it is now realized that IL-2 receptor expression is evanescent after stimulation of the T cell receptor complex (*52*, *53*). Therefore, IL-2 receptors are not detectable on the majority of freshly isolated T cells (Fig. 4). Upon polyclonal activation of the T cell receptor, IL-2 receptors appear asynchronously, and 3 days are required for all of the cells within the population to express IL-2 receptors. Thereafter, the level of IL-2 receptors declines progressively, proliferation eventually ceases, and most of the cells reaccumulate in the resting phase of the cell cycle (G_0 or early G_1) by 12 to 14 days after initiation of the culture.

The immunologic relevance of these findings is understanding that specificity is ensured by the antigen dependence of IL-2 receptor expression (*51–53*). It is a logical extrapolation that IL-2 receptor expression is transient after each stimulation by antigen. As the immune response proceeds and antigen is cleared, transcription of the IL-2 gene and IL-2 receptor genes eventually declines. Simultaneously, another mechanism facilitates the disappearance of IL-2 receptors, one that operates via an effect of IL-2 itself. By binding to high-affinity IL-2 receptors, IL-2 evokes a reciprocal change in the type and character of the binding sites expressed (*54*). Within 1 to 2 hours of IL-2 receptor occupancy, the density of high-affinity IL-2 receptors decreases by as much as 50%. Subsequently, over the course of the ensuing 24 hours, low-affinity p55 β chains gradually increase on the cell surface, eventually exceeding the number of high-affinity IL-2 receptors by a factor of 5 to 10.

Current experiments have provided mechanistic explanations for these phenomena. The membrane half-life ($t_{1/2}$) of unoccupied high-affinity receptors is 150 minutes (*54*), whereas subsequent to IL-2 binding, these receptors disappear ten times more rapidly ($t_{1/2}$ = 15 minutes) (*55*). Consequently, the density of high-affinity IL-2

receptors decreases upon the addition of IL-2 to the cells, primarily as a result of accelerated ligand-mediated internalization. Further study reveals that the p75 α chains determine the rate of internalization of high-affinity α,β heterodimeric receptors (*46*, *55*, *56*). In contrast, the p55 β chains manifest a very slow turnover rate ($t_{1/2} \geq$ 6.5 hours), whether or not they are occupied by IL-2. Such a slow turnover combined with an IL-2–directed transcriptional activation of the p55 β chain gene (*57*) ensures the accumulation of p55 β chains on the cell surface.

These results lead to the conclusion that the intracellular signals generated by the T cell antigen-receptor complex and by IL-2 receptors function to switch membrane IL-2 receptor expression in a reciprocal fashion. Thus, a critical interplay operates between the positive effect of antigen receptor activation and the negative influence of IL-2 receptor triggering, both harmonizing to regulate precisely the density of high-affinity IL-2 receptors and the final proliferative potential of activated T cells (*54*). Such a reciprocal receptor regulatory system provides a fail-safe form of intrinsic control over antigen-initiated but IL-2–dependent T cell clonal expansion. Since high-affinity IL-2 receptors are expressed only upon antigen receptor activation, as antigen is cleared in vivo, the IL-2–directed accelerated internalization of p75 α chains favors the disappearance of functional high-affinity receptors and the return to the state of resting unresponsive cells.

Regulation of T Cell Cycle Progression

Studies during the past 50 years indicate that all cells grow in an essentially identical fashion, including those ranging from bacteria, yeasts, and protozoa to avian and mammalian cells (*58*). Each cell population displays a characteristic mean generation time, but individual cells of the population have appreciably different and variable growth rates. Because of the advances in our understanding of the molecular determinants of T cell growth and because of the development of key reagents such as homogeneous IL-2, radiolabeled IL-2, and monoclonal antibodies reactive with both IL-2 and its receptor, new and fresh approaches to ascertain the determinants of the variability of T cell cycle times became possible. Actually, the knowledge that IL-2 receptor expression is transient after antigen receptor activation led to the utilization of this feature for the development of a new model system for the study of the events important for determining cell cycle progression (*59*).

Examination of the distribution of IL-2 receptor density within a cell population provided the clue that eventually led to a series of experiments demonstrating that only three variables suffice to determine the onset of cell cycle progression—namely, the IL-2 concentration, the IL-2 receptor density, and the duration of the IL-2 receptor interaction (*59*) (Fig. 5). Within an activated T cell population, IL-2 receptor density varies by a factor of 1000 among the individual cells, and the distribution is log-normal, reminiscent of the distribution of cell cycle times. Accordingly, it was natural to wonder whether the cells with the highest density of IL-2 receptors manifest the most rapid cell cycle transit times. Results of several experimental approaches demonstrated convincingly that the IL-2 receptor density and the absolute number of IL-2 receptor interactions determine the time required to transit the G_1 phase of the cell cycle. Thus, a critical threshold of triggered IL-2 receptors must accumulate before an irrevocable move is made to totally replicate DNA. Consequently, at the level of the individual cell, progression to DNA replication and mitosis is a quantal all-or-none decision that is determined precisely by a finite number of IL-2 receptor interactions (*59*).

Even though it was evident that the IL-2 receptor interaction is responsible ultimately for signaling DNA replication and mitosis, what was not clear was whether activation of the T cell antigen–receptor complex promotes movement of the cells through most of G_1 to a point requiring IL-2 for transition to S phase or whether IL-2 itself is responsible for G_1 progression and S phase transition. The synchronized human T cell model system showed that T cell cycle progression was remarkably similar to murine embryonic 3T3 cell growth in that two early phases of the cell cycle can be distinguished (*60*). Activation of the T cell receptor renders the cells "competent" to receive the cell cycle "progression" signals that are provided by IL-2. In this respect, the T cell receptor resembles the platelet-derived growth factor receptor in that antigen-stimulated cells undergo G_0 to G_1 transition but do not progress through G_1 to S phase unless IL-2 is provided. Therefore, the actual molecular mechanism underlying competence, or G_0 to G_1 transition, is now known to be the expression of IL-2 receptors. In contrast, IL-2 promotes a gradual and sustained increase in cell size, the so-called lymphocyte blastic transformation first described by Nowell (*11*), and prepares the cell metabolically for DNA replication. Accordingly, IL-2 is the authentic G_1 progression factor for T cells (*60*).

T Cells as a Model Physiologic Mammalian Cell Growth System

Knowledge of the role of IL-2 in the generation of the T cell immune response and of the separate functions of the T cell receptor and the IL-2 receptor in promoting cell cycle progression make the T cell–IL-2 model system amenable to studies that are fundamental to cell biology, cellular physiology, and the regulation of mammalian gene expression. In this regard, it is especially noteworthy that T cells are available for study from several sources. Thus, T cells can be readily isolated from the circulation and used with minimal manipulation. Moreover, short-term cultures of freshly isolated T cells can be compared with long-term clones of functional antigen-specific normal T cells and neoplastic T cell lines, both of which can be obtained in virtually unlimited quantities. Accordingly, initial biochemical and molecular studies with any of these cells as source material can be reexamined with any of the other T cells, thereby ensuring the veracity and physiological relevance of the results obtained.

With the goal of rendering the short-term culture of T cells as physiologic as possible, recent experiments have focused on determining the external nutrients and serum-derived factors that are essential for optimal T cell growth (*61*). As with prokaryotes, characterization of the critical genetic and biochemical intracellular requirements for eukaryotic cell growth will be feasible only when the external medium is fully defined. Considerable progress toward this goal has already been achieved in that a detailed series of experiments show that IL-2–promoted G_1 progression is completely independent of any of the components supplied by serum (*61*). By comparison, serum components do regulate the G_0 to G_1 transition phase of T cell growth; if serum is excluded entirely from the medium, the expression of IL-2 and the IL-2 receptor genes is delayed markedly (*61*).

The knowledge that IL-2 is the sole requirement for stimulation of the entire series of events recognized as G_1 progression (*59*, *60*) now makes it possible to identify the intracellular pathways that ultimately signal DNA replication. For these studies, molecular genetic approaches have the advantages of extreme sensitivity and specificity for identification of newly expressed genes that may be activated during cell cycle progression. As one example, *c-myb* mRNA transcripts are expressed maximally just at the midpoint of G_1, after 5 hours of IL-2 stimulation (*59*); similar findings have

been obtained by Thompson *et al.* (*62*) in studies of the cell cycle of avian cells. Since c-*myb* is a cellular proto-oncogene that encodes a nuclear binding protein, it will now be important to determine the specificity and function of c-*myb* during cell cycle progression.

Studies of the growth-promoting effects of IL-2 can be compared with studies of the mechanisms whereby IL-2 influences cellular differentiation. Detailed experiments with normal murine B cells, as well as with cloned B lymphoma cells, indicate that IL-2 stimulates the expression of mRNA coding for the secretory form of the μ chain, as well as the J chain, both of which are necessary for the assembly of secreted immunoglobulin M pentamers (*63*). The mechanisms responsible for these effects are now under intense study and may well result from both transcriptional activation and mRNA stabilization.

Immunologic and Therapeutic Implications of the IL-2 Receptor System

The essential nature of IL-2 and IL-2 receptors for the generation of a normal immune response is readily demonstrated by a deficiency in the system. For example, in some of the earliest experiments, immunodeficient athymic mice were found to lack the capacity to produce IL-2 but could respond to it when it was supplied exogenously (*64*). These experimental findings preceded the appearance of the acquired immunodeficiency syndrome, in which the immune system of those afflicted is similar to that of athymic mice; the selective loss of helper T cells eventually results in the inability to produce IL-2 and other lymphokines in response to antigenic challenge. Essentially the same functional defect can also be achieved pharmacologically with the administration of glucocorticoids or cyclosporine, the two most effective immunosuppressive agents known. These two drugs suppress the immune system through a selective inhibition of IL-2 production while having little influence on IL-2 receptor expression (*65*).

Specific approaches to therapeutic immunosuppression that promise to improve the survival of allografts, and also to lead to new therapies for autoimmune diseases, have been developed by capitalizing on the finding that IL-2 receptors are expressed solely by antigen-activated T cells; most unstimulated, resting T cells have no detectable IL-2 receptors. Antibodies reactive with the p55 β chain of the IL-2 receptor specifically prevent cardiac allograft rejection and suppress the development of experimental autoimmune diabetes mellitus and systemic lupus erythematosus as well (*66*). Because of the comparative distributions of the p75 α chain and the p55 β chain of the IL-2 receptor, it is anticipated that when monoclonal antibodies reactive to the p75 α chain become available, even more effective antigen-specific immunosuppression is likely to be achieved.

Bacterial toxin–IL-2 conjugates produced by genetic engineering techniques represent still another approach to antigen-specific immunosuppression. Particularly promising results have recently been reported by Murphy, Kelley, and Strom, and their co-workers, who linked diphtheria toxin to the amino terminal end of human IL-2 (*67*). These workers have attained a remarkable therapeutic index as assessed in antigen-specific immunosuppression in experimental systems, including allograft rejection and delayed-type hypersensitivity models. Their results provide the impetus for further protein engineering approaches with IL-2; antagonists of the IL-2 receptor interaction may provide an effective alternative to actually killing cells expressing IL-2 receptors.

We speculated in our first report that the ability to generate and propagate large numbers of tumor antigen–specific cytotoxic lymphocytes by using a growth factor might represent a practical approach for adoptive immunotherapy (*18*). Now, stimulation of the immune response via the IL-2 receptor is under clinical investigation as a potential new form of immunotherapy for cancer, and some patients have benefited remarkably (*68*). The exact mechanisms responsible for the beneficial effects attained thus far must be elucidated, but they could be explained either by the IL-2–directed clonal expansion of tumor-reactive T cells or by stimulation of natural killer cells.

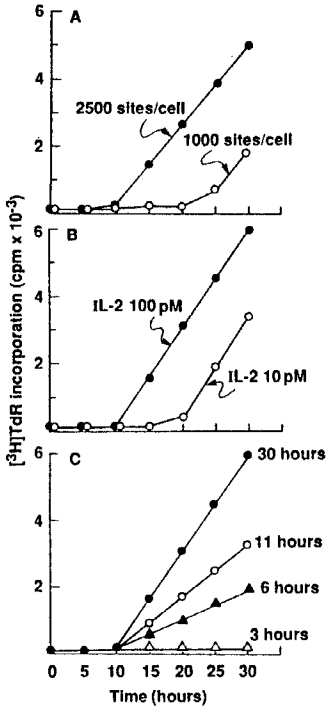

Fig. 5. The variables determining T cell cycle progression. T cells that had been synchronized by a transient proliferation for 2 weeks in IL-2 as depicted in Fig. 4 were used to study the parameters influencing IL-2–promoted G_1 progression to S phase. (**A**) Cell populations manifesting a high density of IL-2 receptors (●) transit G_1 and enter S phase more rapidly than do cells with fewer IL-2 receptors (○). (**B**) By comparison, if the same cell population is exposed to a receptor-saturating IL-2 concentration (●), compared with a concentration expected to saturate only half of the receptors (○), the cells exposed to the higher IL-2 concentration proceed through G_1 most rapidly. (**C**) At least 5 to 6 hours are required for productive IL-2 receptor interactions. Even though IL-2 receptor binding occurs rapidly and comes to a steady state within 15 minutes, even 3 hours of IL-2 exposure is insufficient to stimulate G_1 progression and S-phase transition. Moreover, with longer intervals of IL-2 exposure, a progressive increase in the proportion of cells that begin S phase can be detected, as monitored by tritiated thymidine ([^{3}H]TdR) incorporation. Identical results were obtained when flow cytometry and quantitative DNA staining with propidium iodide were used. [Redrawn from D. A. Cantrell and K. A. Smith (*59*), with permission]

Others have reported on the use of IL-2 as an adjuvant for stimulating the immune response to vaccines (*69*). Thus far, there are no safe, nontoxic adjuvants for use in humans that compare with the effectiveness of Freund's complete adjuvant as used in experimental animals. IL-2 may eventually become a widely applied adjuvant, particularly as new generation vaccines based on recombinant proteins and synthetic peptides are developed. A particularly novel approach has recently been found promising, especially for immunosuppressed hosts; the IL-2 cDNA coding region has been inserted into vaccinia virus together with antigen-coding sequences. The vaccinia-directed IL-2 produced allows for an effective immune response (*69*).

From all of these data, it is evident that our understanding of the IL-2 receptor interaction and its mechanism of stimulation of antigen-specific T cell clonal expansion is pivotal, making possible the formulation of new rational approaches to two major immunotherapeutic goals—that is, controlled, specific immunosuppression and immunoenhancement. Thus, traditional pharmacologic principles can now be applied to the heretofore rather impenetrable immune system to create agents that modulate IL-2 production and IL-2 action. These then are the developments that lead to the anticipation of the discovery of selective antagonists and agonists that operate via the IL-2 receptor, so that truly effective medicinal intervention will eventually become a reality in a wide variety of disorders of the immune system.

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70. I am grateful to M. Landy for editorial comments. This work was supported in part by grant CA17643 from the National Cancer Institute, by grant 1715 from the Council for Tobacco Research, U.S.A., Inc., and by a grant from the Eli Lilly Corporation.