The Multichain Interleukin-2 Receptor: A Target for Immunotherapy

Moderator: Thomas A. Waldmann, MD; Discussants: Ira H. Pastan, MD; Otto A. Gansow, PhD; Richard P. Junghans, PhD, MD

Activation of resting T-lymphocytes induces synthesis of interleukin-2 (IL-2) and expression of cell surface receptors for this lymphokine. In contrast to resting normal T-cells that do not express high-affinity IL-2 receptors (IL-2R), abnormal T-cells of patients with leukemia-lymphoma, certain autoimmune disorders, and individuals rejecting allografts express this receptor. Exploiting this difference in receptor expression, antibodies to the IL-2 receptor have been used effectively to treat patients with leukemia and lymphoma. One approach is to use monoclonal antibodies produced in mice; the disadvantage is that they are highly immunogenic. In an effort to reduce the immunogenicity of the mouse monoclonal antibodies, monoclonal-antibody-mediated therapy has been revolutionized by generating humanized antibodies produced by genetic engineering in which the molecule is human except for the antigen-combining regions, which are retained from the mouse. Further, to increase its cytotoxic effectiveness, the monoclonal antibody has been armed with toxins or radionuclides. Alternatively, IL-2 itself has been linked to a toxin to kill IL-2 receptor-bearing cells. Thus, IL-2 receptor-directed therapy provides a new method for treating certain neoplastic diseases and autoimmune disorders and for preventing allograft rejection.


Dr. Thomas A. Waldmann (Chief, Metabolism Branch, National Cancer Institute): Chemotherapeutic agents have cured some types of human cancer; however, many cancers are either initially unresponsive or subsequently acquire resistance to chemotherapy. The development of hybridoma technology to produce monoclonal antibodies by Köhler and Milstein (1) captured the imagination of medical scientists and rekindled interest in the use of antibodies as agents to treat patients with cancer. The initial use of monoclonal antibodies was relatively ineffective, however, with only 23 partial and 3 complete remissions reported among 185 patients in 25 clinical trials (2).

Many factors have been responsible for this low therapeutic efficiency. One factor is that the murine monoclonal antibodies are foreign proteins that are immunogenic to humans. An even more serious problem is that most of the monoclonal antibodies used are not effective at killing human neoplastic cells. Further, in most cases the antibodies are not directed against a vital structure present on the surface of malignant cells, such as a growth factor receptor, which is required for tumor cell proliferation and survival.

We have redressed this issue by using the interleukin-2 receptor (IL-2R) as a target for immune intervention (3, 4). The scientific basis for this approach is that resting normal cells do not express IL-2R. Rather, this receptor is expressed by a proportion of the activated cells in certain forms of lymphoid neoplasia, in select autoimmune diseases, and in allograft rejection. As we shall discuss, therapeutic trials have been initiated to exploit this difference in IL-2R expression, using an unmodified monoclonal antibody to the IL-2R, called anti-Tac, as well as antibodies armed with toxins or radionuclides to increase their ability to kill the target abnormal cells. Furthermore, bifunctional antibodies were produced with dual specificities: one toward IL-2R-expressing target cells and one toward killer cells in order to retarget killer cells to IL-2R-expressing cells. Finally, in order to reduce its immunogenicity and increase its effector function, the mouse anti-IL-2R monoclonal antibody was converted by genetic engineering into a molecule that is entirely human immunoglobulin G (IgG), except for the small antigen-binding regions that are retained from the mouse antibody.

Structure and Function of the Multi-subunit Interleukin-2 Receptor

To function as effector cells, T cells must change from a resting to an activated state. The sequence of events involved in the activation of T-cells begins when a foreign pathogen encounters the antigen-specific receptor on the surface of resting T-cells. This antigen-stimulated activation of these resting T-cells induces the synthesis of the 15-kDa lymphokine IL-2. To exert its biologic effect, IL-2 must interact with specific high-affinity membrane receptors (3-8). Resting cells do not express high-affinity IL-2R, but they are rapidly expressed on T cells after activation with antigen or mitogen (3, 9).

There are three forms of cellular receptors for IL-2: one with a very high affinity (10^11/M), one with an
intermediate affinity (10^9/M), and another with a much lower affinity (10^6/M) for IL-2. We have used monoclonal antibodies and radiolabeled IL-2 in cross-linking studies to characterize chemically the multiple subunits of this receptor (Figure 1). Initially, a monoclonal antibody (anti-Tac) that reacts with the interleukin-2 binding site of a 55-kDa IL-2R protein (now termed IL-2Rα) was identified (10, 11). The receptor protein identified by anti-Tac was characterized as a glycoprotein with an apparent molecular mass of 55 kDa. Using cross-linking methods, we defined a second 70- to 75-kDa (anti-Tac) that reacts with the interleukin-2 binding site of this receptor (12, 13). We proposed a multi-subunit model for the high-affinity IL-2R in which both IL-2Rα and IL-2Rβ proteins are associated in a receptor complex (12, 13) (Figure 1). In an independent study, Sharon and colleagues proposed a similar model (14). A series of non-IL-2 binding peptides are associated with IL-2Rα and IL-2Rβ in a multi-subunit IL-2 receptor complex (15-19).

The IL-2Rβ subunit is a member of a new family of growth and differentiation factor receptors, the hematopoietin receptor superfamily, which includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), prolactin, growth hormone, and erythropoietin (20, 21). The shared features of the receptors in the hematopoietin receptor superfamily include four conserved cysteine residues located in the N-terminal half of the extracellular ligand-binding domain and a Trp-Ser-X-Trp-Ser motif (WSXWS) located just outside the membrane-spanning domain.

Interleukin-2 Receptor Expression in Malignancy or Autoimmune Disorders

Resting T-cells, B-cells, or monocytes in the circulation do not display the IL-2α receptor chain. However, most T- and B-lymphocytes can be induced to express this receptor subunit. Further, Rubin and coworkers (22) showed that activated normal peripheral-blood mononuclear cells and certain lines of T- or B-cell origin release a soluble form of the IL-2Rα into the culture medium and showed that normal individuals have measurable amounts of IL-2Rα in their plasma. The determination of plasma levels of such IL-2Rα provides a valuable noninvasive approach for analyzing both normal and disease-associated lymphocyte activation in vivo.

In contrast to the lack of IL-2Rα chain expression in normal resting mononuclear cells, this receptor peptide is expressed by a proportion of the abnormal cells in certain forms of lymphoid neoplasia, in select autoimmune diseases, and in allograft rejections. That is, a proportion of the abnormal cells in these diseases expresses surface IL-2Rα peptide. Further, the serum concentration of the soluble form of the Tac peptide is elevated in the plasma of such individuals (4, 22). In terms of neoplasia, certain T-cell, B-cell, monocytic, and even granulocytic leukemias express the IL-2Rα chain. Specifically, virtually all of the patients with human T-cell lymphotrophic virus-I (HTLV-I)-associated adult T-cell leukemia constitutevly express very large numbers of IL-2Rα (23, 24). Similarly, a proportion of patients with cutaneous T-cell lymphomas expresses the Tac peptide (23, 25). Further, the malignant B-cells of virtually all patients with hairy cell leukemia and a proportion of patients with large and mixed-cell diffuse lymphomas express IL-2Rα (26). The IL-2Rα is also expressed on the Reed-Sternberg cells of patients with Hodgkin disease and on the malignant cells of patients with true histiocytic lymphoma (25). Finally, a proportion of the leukemic cells of patients with chronic and acute myelogenous leukemia express the Tac antigen (IL-2Rα).

Autoimmune diseases may also be associated with disorders of Tac antigen expression (27, 28). A proportion of the mononuclear cells in the involved tissues expresses IL-2Rα chain, and the serum concentration of the soluble form of this chain is elevated. Such evidence for T-cell activation and disorders of Tac antigen expression appears in more than 15 autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus, scleroderma, pulmonary sarcoidosis, and HTLV-I-associated tropical spastic paraparesis. Finally, the Tac peptide is also expressed by the activated
lymphocytes in recipients of renal, hepatic, and cardiac allografts that are reacting to the foreign histocompatibility antigens expressed on the donor organs (3, 27).

Disorders of Interleukin-2 Receptor Expression in HTLV-1-associated Adult T-Cell Leukemia

A distinct form of mature T-cell leukemia defined by Uchiyama and coworkers was termed adult T-cell leukemia (29). The retrovirus HTLV-1 was shown to be the primary causative agent in this leukemia (30). Adult T-cell leukemia is a malignant proliferation of mature CD4+ expressing T cells that infiltrate the skin, lungs, and liver. Cases of adult T-cell leukemia are associated with hypercalcemia and an immunodeficiency state and usually have a very aggressive course with a mean time to death of 20 weeks. The leukemic cells that we and others have examined from patients with HTLV-1-associated adult T-cell leukemia express high- and low-affinity IL-2R, including the IL-2R α chain (23, 24). An analysis of HTLV-1 and its protein products suggests a potential mechanism for this association between HTLV-1 and the constitutive IL-2R α expression. The retrovirus HTLV-1 encodes a 42-kDa protein, now termed tax, that is essential for viral replication (31, 32). The tax protein, encoded by this retrovirus, also plays a central role in indirectly increasing the transcription of host genes, including the IL-2 and especially the IL-2R α receptor genes involved in T-cell activation and HTLV-1-mediated leukemogenesis.

Interleukin-2 Receptor α as a Target for Therapy in Patients with HTLV-1-associated Adult T-Cell Leukemia

Unmodified Anti-Tac Monoclonal Antibody

The HTLV-1-induced adult T-cell leukemia cells constitutively express the IL-2R α chain identified by the anti-Tac monoclonal antibody, whereas normal resting cells do not. This observation provided the scientific basis for IL-2R-directed immunotherapy with this monoclonal antibody. Interleukin-2 receptor-directed immunotherapeutic agents could theoretically eliminate IL-2R α-expressing leukemic cells or abnormally activated T-cells involved in other disease states while retaining the Tac nonexpressing normal T-cells and their precursors that express the antigen receptors for T-cell immune responses. In our initial studies, we administered intravenous unmodified murine anti-Tac to patients with adult T-cell leukemia (33). The leukemic cells of each patient with adult T-cell leukemia reacted with anti-Tac. Our goal was to inhibit the interaction of IL-2 with its growth factor receptor expressed on the malignant cells. The 20 patients treated in this study did not have untoward reactions related to the immunotherapy and did not have a reduction in the normal formed elements of the blood. Only patients undergoing a remission produced antibodies to the monoclonal antibody. Seven of the 20 treated patients had transient mixed (1), partial (3), or complete remissions (3), lasting from 1 to more than 24 months after anti-Tac therapy. This was assessed by elimination of measurable skin and lymph nodal disease, normalization of serum calcium levels, and routine hematologic and phenotypic tests of circulating cells. Further, elimination of clonal malignant cells was shown by molecular genetic analysis of HTLV-1 proviral integration and the T-cell antigen receptor gene rearrangements. Thus the use of a monoclonal antibody that prevents the interaction of IL-2 with its growth factor receptor on adult T cells-leukemia cells provides a rational approach for treating this malignancy. Indeed, Maeda and coworkers (34) have presented evidence for the IL-2-dependent expansion of leukemic cells in adult T-cell leukemia in approximately 20% of cases. In most cases of the aggressive phase of adult T-cell leukemia, however, the leukemic cells no longer produce IL-2 nor do they require IL-2 for their proliferation. In this phase of the disease, the patients may not be responsive to unmodified anti-Tac therapy. Nevertheless, such cells continue to display the Tac protein.

Thus there is still a difference between the normal cells and the malignant cells that can be explored in treatment. To continue to take advantage of this difference and to improve the effectiveness of IL-2R-directed therapy, the antibody has been armed with cytotoxic agents. For example, Pseudomonas exotoxin has been coupled to anti-Tac to deliver a cytotoxic substance directly to the target cancer cells and abnormal T-cells.

Recombinant Toxins Directed toward the Interleukin-2 Receptor

Dr. Ira H. Pastan (Chief, Laboratory of Molecular Biology, National Cancer Institute): We have used a potent cytotoxic agent, a toxin, to kill cells selectively. The strategy is simple: Select from nature one of the

<table>
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<th>Table 1. Activity of Various Toxins</th>
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</tr>
<tr>
<td>Anti-Tac-PE</td>
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<tr>
<td>Anti-Tac-PE-40</td>
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<tr>
<td>IL-2-PE-40</td>
</tr>
<tr>
<td>Anti-Tac(Fv)-PE-40</td>
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* ID₅₀, the dose required to inhibit protein synthesis by 50%.
† Specificity window, the factor defining the difference between dose required for therapeutic effect and that causing toxicity.
‡ LD₅₀, the dose that is lethal to 50% of the animals.
§ For HUT-102 cell line.
¶ For KB cell line.
most toxic proteins available, a toxin that kills all sorts of cells. We selected *Pseudomonas* exotoxin. Next, modify the toxin so that it will no longer indiscriminately kill all cells, but will instead kill only the cells of interest.

*Pseudomonas* exotoxin is made by *Pseudomonas aeruginosa*, a common soil organism. The protein toxin is secreted as a single polypeptide chain of 66 000 molecular weight, and it kills cells by inhibiting protein synthesis. It is an enzyme that catalyzes the adenosine diphosphate (ADP) ribosylation and inactivation of Elongation Factor II, a factor that is required for protein synthesis. When protein synthesis stops, the cell dies. The potency of this exotoxin makes it interesting to us. It is a very potent catalyst that irreversibly arrests protein synthesis. Thus, a single molecule within the cytosol is sufficient to kill a cell.

The steps involved in cell killing are as follows: *Pseudomonas* toxin binds to a receptor present on the surface of almost every type of vertebrate cell. Next, the toxin enters an endocytic vesicle. The pH in the vesicle becomes acidic and, in a little understood reaction, some of the toxin molecules that had entered the cell are proteolytically cleaved to a 37-kDa form that is translocated into the cytosol and ADP ribosylates Elongation Factor II. Our task was to harness this toxin, modify it, and to make it kill cells while it ignored other cells.

We began working several years ago with the anti-Tac antibody, coupling it chemically to *Pseudomonas* toxin to make an immunotoxin termed anti-Tac-*Pseudomonas* toxin. We tested anti-Tac-*Pseudomonas* toxin on a Tac expressing malignant T-cell line (HUT-102) from a patient with HTLV-I-associated leukemia. Anti-Tac-*Pseudomonas* toxin killed such cells, and the killing was relatively specific (35, Table 1). The window of specificity was about a factor of 100.

We tested this reagent in monkey toxicity studies and then in patients with adult T-cell leukemia. We found that we could give only a few milligrams a day to patients without producing undesirable liver damage, because we had not sufficiently changed the toxin so that it would no longer bind to normal cells, such as liver cells, which are very sensitive to *Pseudomonas* toxin.

At this time, McKay and colleagues had worked out the three-dimensional structure of *Pseudomonas* toxin (36). Their studies showed that the native toxin was made up of three major domains. We were interested in knowing the function of these domains because we thought that if we could identify and remove the domain responsible for binding to normal human tissues, the molecule could be redesigned by genetic engineering to kill cells more specifically. Therefore, we isolated the *Pseudomonas* toxin gene and expressed the whole toxin or pieces of the toxin in *Escherichia coli* and determined the function of each of the three domains. The domains are called I, II, and III (37). We found that domain I was responsible for cell binding. Domain II had the properties of a cell-penetrating protein; it helped the toxin cross the membrane into the cytosol. Domain III was the ADP ribosylating enzyme responsible for cell killing. By this analysis, then, we had a protein made up of three domains, each with a separate function: I, cell binding; II, membrane penetration; and III, ADP ribosylation. Next, we deleted the binding domain and made a modified toxin protein called PE-40, a 40 000 molecular weight form of *Pseudomonas* toxin, which has only the translocating and ADP ribosylating domains. We reasoned that PE-40 should have very low toxicity toward cells or animals because it could not bind to the cell surface.

Table 1 shows that the ID₅₀, the quantity required to kill 50% of the cells for anti-Tac-*Pseudomonas* toxin on HUT-102 cells, is 2 ng/mL, whereas on a KB carcinoma cell line that has no Tac antigen, the ID₅₀ is about 200 ng/mL. Thus, the window of specificity is a factor of 100. Let us now look at anti-Tac-PE-40. On HUT-102...
cells the ID₅₀ is about 10 ng/mL; thus, it is a little less active than anti-Tac-Pseudomonas toxin. But the important finding was that the toxicity on KB cells was gone (see Table 1). Further, the toxicity in mice was reduced by 50-fold so we can now give 50-fold more (38). Thus, by chemically replacing domain I with the anti-Tac antibody, we have made a molecule that is very active in cell culture. We anticipate that we can give up to 50 mg/d to a patient before reaching a toxic level.

Therefore, one way to attack the problem is to delete domain I so that the modified Pseudomonas toxin can no longer bind nonspecifically to cells, to make the recombinant molecule in E. coli, and to couple it chemically to antibodies. The resultant molecule, of course, will only bind to the Tac peptide IL-2R α. We next took the gene encoding PE-40, and, by genetic engineering, fused a cDNA that encodes IL-2 to the PE-40 gene to produce a chimeric toxin (39). The chimeric toxin, IL-2-PE-40, binds to cells that have IL-2R, and specifically kills those cells (see Table 1). We chose the name IL-2-PE-40 for the chimeric toxin to indicate the molecular weight of the toxin portion (40 000).

On HUT-102 cells, the ID₅₀ (the quantity required to inhibit protein synthesis by 50%) of IL-2-PE-40 is 3 ng/mL. On a cell line without IL-2Rs, it is nontoxic (see Table 1). We have checked IL-2-PE-40 not only against mouse cancer cell lines displaying IL-2R, but also against mouse splenocytes that have been activated either by CON-A or by allogenic mechanisms in a mixed leukocyte reaction (40). Although IL-2-PE-40 does not kill resting T cells, it does kill both CON-A blasts and allogenically activated monocytes because of the IL-2R they acquire when activated.

Recently, we have begun animal experiments with IL-2-PE-40. This substance is not very toxic; the LD₅₀ in mice is about 50 μg (see Table 1). We have done some experiments with Robert Kozak that look at an IL-2R-bearing lymphoma in mice; these experiments appear promising because we see tumor suppression. With Drs. Kirkman and Barrett at Brigham and Women’s Hospital, we have examined a cardiac allograft model, in which a heart from a B10 • BR mouse is transplanted into a recipient C57 • B10 mouse. Groups of five animals were treated daily for 10 days with different agents (Figure 2). Some groups of animals received increased amounts of IL-2-PE-40, others received control proteins. On day 10, treatment was stopped and graft survival was monitored (41). In mice receiving IL-2-PE-40 at 0.5 μg/g, all of the grafts survived; PE-40 alone had little or no effect. We speculate that we have prolonged allograft survival by eliminating the activated cytotoxic cell clones that are involved in graft rejection.

We have also conducted experiments with Drs. Case, Lafyatis, and Wilder, also at NIH, using a rat adjuvant arthritis model (42). In this system, Lewis rats are injected with Mycobacterium butyricum in oil. The experimental group receives IL-2-PE-40 at 0.5 μg/g body weight every 12 hours for 10 days; the control groups receive phosphate-buffered saline (PBS). The animals are examined daily for joint swelling. Figure 3 summarizes the development of arthritis in the joints of these animals as a function of days after initiation of the experiment. In the IL-2-PE-40 treated group, there is a decrease in the arthritis index score, whereas the various control substances including PE-40 (data not shown) or phosphate-buffered saline have little or no effect. It appears that significant effects can be shown in animals, and we attribute these effects to the elimination of activated T-cells. Recently, it has been possible to make another type of agent that kills cells that have IL-2R. This is a chimeric toxin in which an antibody-combining site of anti-Tac is fused to PE-40. These single-chain immunotoxins appear much more active than the other agents directed at the IL-2R of human and subhuman primates (see Table 1 and reference 43).

It is also possible to use this technology to target the toxin to kill cells with epidermal growth factor receptors using transforming growth factor alpha (44), to kill human immunodeficiency virus (HIV)-infected cells by making a CD4 fusion protein (45), and to kill some myeloma cells by using IL-6 to target the toxin (46). Now that we have material that is active in animals, we are working with several industrial groups to make large amounts of drugs so that we can carry out studies in patients with leukemia, cancer, and other incurable diseases.

**Radiometal Chelate—Conjugated Monoclonal Antibodies for Use in Radioimmunodiagnosis or Radioimmunotherapy**

Dr. Otto A. Gansow (Head, Inorganic and Radioimmunochemistry Section, Radiation Oncology Branch, National Cancer Institute): There is a difference between our approach to using the IL-2R as a target for a radiation-based immunotherapy (47) and the approaches that use as therapeutic modalities various drugs or toxins linked to antibody. Because chemotherapeutic agents and toxic proteins are, in general, effective only inside the cell, it follows that for therapy to be success-
ful, the immunoprotein to which they are attached must be transported to within the cell membrane. However, most monoclonal antibodies, including IL-2R-directed anti-Tac, are not readily modulated into the cell (48). The advantage of using a radioactive nuclide as a therapeutic agent is that it is cytotoxic over a measurable range from the source (49). Thus, for therapy with antibody-delivered radionuclides, all that is required is that the radioisotope be localized at the surface of the target cell. Moreover, use of isotopes capable of being imaged for tumor targeting can facilitate estimation of the radiation dose provided to the tumor target (50). We have focused our efforts on developing the scientific knowledge to produce antibody conjugates of those radionuclides thought to be suitable for radioimmunomaging and radioimmunotherapy.

Nuclear chemistry has provided a selection of radioisotopes that could be linked to immunoproteins (Table 2). Only a few elements, such as iodine, can directly react with amino acid residues of proteins. Unfortunately, none of the radioiodine nuclides are optimal either for scanning of tumors or for therapy (51). The labeling of immunoproteins with the metallic radioisotopes required us to synthesize chemically modified organic molecules, called chelating agents (CAs) or ligands, with various chemical structures (52). Ligands were selected because they exhibit exceedingly high thermodynamic stability constants for trivalent metal ions (commonly K > 10^23) and because they are kinetically inert and do not release the metal from within metal chelate structures. It is crucial that the metal not be released in vivo from inside the antibody-linked chelate and so lead to radiation concentration in such natural metal binding sites such as bone, liver, or kidney yielding unacceptable toxicity (53). We reported a few years ago (54) that gamma-camera images of tumors may be obtained after injection of indium-111 (111In) metal chelates linked to monoclonal antibody. This shows the potential for radioimmunotherapy to treat leukemia and other blood-borne diseases, because in principle, cytotoxic, particulate, beta, or alpha radiation might be similarly concentrated to effect highly localized radiation therapy. Shortly after reporting our work, we began studies with the monoclonal antibody anti-Tac that targets IL-2R expressed in high concentrations on the surface of the malignant cells of patients with adult T-cell leukemia (55).

Particulate radiation is thought to mediate cytotoxicity by the reaction of free radicals formed when electrically charged beta or alpha radiations traverse the cell nucleus, causing breaks in DNA and disrupting replication. Alpha particles passing through tissue create dense tracks of ionizing radiation, whereas tracks of beta particles are sparse by comparison (56). Because the number of binding sites of antibodies on cells is limited, alpha emitters might be more useful for radioimmunotherapy than beta particles. Therefore, the first priority of our chemical studies was the linkage of an alpha emitter, bismuth-212 (212Bi), to immunoproteins (57). By using a diethylene-triaminepentaacetic acid anhy-

<p>| Table 2. Radionuclides Suitable for Imaging, Therapy, or Both |
|----------------------|--------|--------------|----------|-------|</p>
<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half-Life</th>
<th>Particle</th>
<th>Maximum Energy*</th>
<th>Source†</th>
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<tr>
<td><strong>Imaging</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Indium (111In)</td>
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<td>γ</td>
<td>0.173, 0.245</td>
<td>Accelerator +</td>
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<tr>
<td>Technetium (99mTc)</td>
<td>6.0</td>
<td>γ</td>
<td>0.140</td>
<td>Generator +</td>
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<tr>
<td>Gallium (67Ga)</td>
<td>78.3</td>
<td>γ</td>
<td>0.093, 0.184, 0.296</td>
<td>Accelerator +</td>
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<tr>
<td>Rhenium (186Re)</td>
<td>90.6</td>
<td>γ</td>
<td>0.137</td>
<td>Nuclear reactor −</td>
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<tr>
<td>Iodine (131I)</td>
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<td>Lead (203Pb)</td>
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<td>Accelerator +</td>
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<td><strong>Therapy</strong></td>
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</tr>
<tr>
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<td>γ</td>
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<td>γ</td>
<td>2.116</td>
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<td>Bismuth (212Bi)</td>
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<td>Samarium (153Sm)</td>
<td>46.8</td>
<td>γ</td>
<td>0.727</td>
<td>Accelerator +</td>
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* Average energies are approximately one third of the maximum energy values. There are multiple gamma ray or alpha particle emissions in certain radionuclides; the maximum energies for these emissions are listed as separate entries.
† Availability—whether more (+) or less (−) obtainable for general use.
Iodine-131- and yttrium-88-labeled anti-Tac-chelating agents (CA) were studied in athymic nude mice. These mice were injected in the tail vein with 1 μCi of each radionuclide-labeled, CA-coupled anti-Tac. Plasma clearance of anti-Tac-CA-SCN-Bz2 and anti-Tac-CA-CaDTPA was similar to clearance of I-labeled unmodified anti-Tac or I-labeled anti-Tac, indicating minimal elution of 89Y from the complex. In contrast, plasma clearance of anti-Tac-CA-88Y using three other agents (1B-EDTA, CaDTPA, and 2B-DTPA) was much faster than the clearance of their 131I-labeled counterparts, indicating 89Y was rapidly eluted from the complex. (Reproduced from reference 61.)

Figure 4. Plasma clearance. Iodine-131- and yttrium-88-labeled anti-Tac-chelating agents (CA) were studied in athymic nude mice. These mice were injected in the tail vein with 1 μCi of each radionuclide-labeled, CA-coupled anti-Tac. Plasma clearance of anti-Tac-CA-SCN-Bz2 and anti-Tac-CA-CaDTPA was similar to clearance of I-labeled unmodified anti-Tac or I-labeled anti-Tac, indicating minimal elution of 89Y from the complex. In contrast, plasma clearance of anti-Tac-CA-88Y using three other agents (1B-EDTA, CaDTPA, and 2B-DTPA) was much faster than the clearance of their 131I-labeled counterparts, indicating 89Y was rapidly eluted from the complex. (Reproduced from reference 61.)

In summary, the choice of chelating agent used to radiolabel monoclonal antibodies with various metallic radionuclides useful for radioimmunotherapy is of major importance. Unstable metal chelates release cytotoxic radioactivity that localizes in vivo and provides sub-
Bifunctional and “Humanized” Chimeric Anti-Interleukin-2 Receptor Antibodies

Dr. Richard P. Junghans (Metabolism Branch, National Cancer Institute): In the preceding discussions we have glimpsed the potential for antibody therapies that direct toxin or radioisotope to targeted cells. These approaches, however, tacitly abandon the immune system and its many cooperative functions as being inadequate to the task. I wish to retake some of this ground by re-examining the mechanisms by which the immune system works, or does not work, and try to reharness those mechanisms to combat human disease. Two major problems hamper the effectiveness of monoclonal antibodies in human therapy. One problem is immunogenicity: Most monoclonal antibodies are mouse proteins that are neutralized by human anti-mouse antibodies as soon as they develop, usually within the first month of therapy. The second problem is that such antibodies are often ineffective at recruiting host effector functions, namely, antibody-dependent, cell-mediated cytotoxicity.

In antibody-dependent cellular cytotoxicity, target cells coated with antibody attract effector cells equipped with Fc receptors that bind to the Fc region of IgG and release cytolysins that lead to cell killing. There are problems with antibody-dependent cellular cytotoxicity, but they are relatively amenable to manipulation. First, effector-target cell conjugate formation, which is inefficient, must occur before killing can take place. The Fc receptor of killer cells has a relatively low affinity for cell-bound antibody, which must also compete with high levels of circulating immunoglobulin. Second, mouse antibodies, for the most part, do not work well in antibody-dependent cellular cytotoxicity with natural human targets.

To address these questions, we followed two strategies using bifunctional and chimeric antibodies. With bifunctional antibodies, we sought to improve conjugate formation by creating molecules that have dual specificities: one to the target cell and one to the killer cell. The purpose of such molecules is to direct the killer cells—both killer (K) cells and T cells—to targets based on the antibody specificity in a function termed effector cell retargeting. In the case of K cells arising from the large granular lymphocyte population, which normally mediates antibody-dependent cellular cytotoxicity, the bifunctional antibody is able, through its anti-FcR moiety, to substitute a high-affinity antigen-antibody interaction with a resultant marked improvement in conjugate formation (64). In the case of T cells, the antibody is able to recruit an entirely new cell class into antibody-directed killing. With the binding of anti-T3 to the CD3 antigen, the T cell is stimulated into a killing mode, bypassing the normal major histocompatibility complex and antigen-specific restrictions of T-cell killing. Two types of molecules accomplish this purpose. For the sake of this discussion, we will call the first type of construct “heteroconjugate,” complete immunoglobulin molecules that have been chemically cross-linked. The heteroconjugate is a dimer or trimer and carries within it two different specificities. The second type of construct we call “bispecific antibody.” This is made up of two half-molecules. It is monomeric and produced by hybrid hybridomas or, in vitro, by disulfide exchange. For our studies we used the HUT-102 cell line as a model target. This is a human leukemia T-cell line that expresses about 200 000 receptors identified by anti-Tac per cell, and expresses about one to two million HLA-B17 molecules per cell. It is T3 (CD3) negative, and Fc receptor gamma (CD16) negative. Figure 5 shows results of effector cell retargeting. In this strategy, we mix antibody-coated targets with peripheral blood mononuclear cells (with or without IL-2 activation) in varying ratios in a 4-hour chromium release assay. Both heteroconjugates—anti-Tac with anti-FcR (CD16) and anti-Tac with anti-T3 (CD3) (produced for us by Dr. David Segal)—showed potent cell killing, above that even with the control positive human antiserum to the appropriate histocompatibility locus antigen. The native mouse anti-Tac was consistently negative in the assay (Figure 5). Anti-CD3 or anti-CD16, alone or mixed with anti-Tac, are inert in the assay in contrast to the heteroconjugates (data not shown).

Figure 5. Cellular cytotoxicity with bifunctional antibodies. Tac-expressing HUT-102 T human target cells are coated with antibody and incubated with an excess of human peripheral blood mononuclear cells in a 4-hour chromium-51 release assay. Anti-T17 is a positive control human anti-HLA antiserum that is reactive with HUT-102. Anti-Tac does not mediate antibody-dependent cellular cytotoxicity and is consistently negative in the assay. Anti-Tac × anti-CD16 and anti-Tac × anti-CD3 are heteroconjugate antibodies prepared by in-vitro cross-linking.
Although these in-vitro observations are encouraging, there are reasons not to use heteroconjugate antibodies. As molecular aggregates, they would have reduced in-vivo survival and reduced tissue penetration. Monomeric bispecific antibodies, however, avoid these problems. We have used two approaches to produce monomeric bispecific antibodies: First, we created bispecific antibodies in vitro with chemical methods; second, we prepared hybrid hybridomas. Physical chemists know that immunoglobulin can be reduced, denatured in acid, and reassembled under oxidizing conditions to generate mixed immunoglobulins (65). We created several such hybrids. Binding studies demonstrated bifunctional products and a modest autologous H:L preference in reassembly. However, these antibodies did sustain damage after reassembly from acid; only 1% or 2% of the product was bifunctionally active as opposed to the 15% we predicted (66). Yet, even this crude material showed killing activity in effector cell retargeting.

We are exploring methods to improve recovery of active antibody from the in-vitro assembly that could be of general use and that avoid the demands of hybridoma technology. In parallel, we have prepared hybrid hybridomas by fusion and selection techniques. The supernatants of these hybrid hybridomas showed effector cell retargeting for both anti-CD3-anti-Tac and anti-CD16-anti-Tac bispecific antibodies.

Next, I want to discuss chimeric human-mouse antibodies, which have been humanized to various degrees by recombinant DNA technology. These antibodies were devised to address the problems of immunogenicity by reducing the murine component of the antibody. In addition, we hoped that exchanging human for mouse constant regions might yield improvements in effector function. Chimeric and "hyperchimeric" antibodies were produced (Figure 6), the former with complete exchange of constant regions and the latter with replacement of variable framework regions as well, retaining only the mouse hypervariable segments from the original antibody essential for antigen recognition (67). The hypervariable segments in anti-Tac are 6 to 19 amino acids in length. The hyperchimeric molecule (called anti-Tac-H) is thus more than 90% human. In collaboration with Dr. Cary Queen of Protein Design Labs in Palo Alto, California, several constructs were prepared, of which a representative few are shown in Table 3.

We asked three questions about these antibodies in our preclinical evaluations. First, do they maintain their affinity for antigen; second, do they continue to suppress IL-2 activation of T cells, which is critical to our transplant experiments; and third, is antibody-dependent cellular cytotoxicity, which is absent in the parental mouse antibody, obtained? To the first question, the answer was invariably yes: All constructs bound antigen. The human IgG, and IgG, constructs showed comparably high levels of affinity, whereas the affinity of the hyperchimeric antibody was reduced by a factor of 3, but still maintained high-affinity (3 x 10^9/M) binding (67). The answer to the second question was also yes. The chimeric antibodies blocked T-cell activation in antigen stimulation and mixed-leukocyte reaction assays (68). Figure 7 shows tetanus toxoid stimulation of sensitized T-cells and comparable levels of suppression of anti-Tac by the G1 and G3 chimeras and by the hyperchimeras. Finally, the human G1 chimera and the G1 hyperchimera were both cytotoxic in the antibody-dependent cellular cytotoxicity assays, whereas the native mouse anti-Tac is consistently inactive (68). The human G3 chimera was negative, correlating with the results of other investigators showing G3 chimeras to be less active in antibody-dependent cellular cytotoxicity (69, 70). The activity with the chimeric and hyperchimeric antibodies was significantly stimulated with IL-2 treatment of effector cells.

In summary, bifunctional and chimeric antibodies are two approaches to improve the therapeutic profile of monoclonal antibodies. Bifunctional versions of anti-Tac perform effector cell retargeting with improved cell killing. This included not just the Fc receptor-positive effector cells that normally participate in antibody-dependent cellular cytotoxicity, but the cytotoxic T-lymphocytes as well. A combination of such bispecific antibodies might produce summative effects in vivo. Humanized versions of anti-Tac maintained their capacity to bind to antigen targets to block IL-2-mediated T-cell activation that is critical to our transplant experiments. These chimeric antibodies provide new capabilities to perform antibody-dependent cellular cytotoxicity, which could have a major effect on in-vivo therapies. Initial reports from trials using the only other hyperchimeric antibody and a simple chimeric construct are encouraging in that an immune response may be avoided with anti-Tac-H as well (71, 72). A merging of technologies could yield humanized bifunctional antibodies for maximum in-vivo survivability and maximum recruitment of host effector functions. Together, these technologies offer renewed hope for antibody-based immunotherapies, with potentially great impact on their effectiveness in the treatment of cancer and immune disorders.

Overview of Interleukin-2 Receptor Directed Therapy: Present and Future Directions

Dr. Thomas A. Waldmann: We have discussed the application of basic biologic insights derived from the laboratory to the development of rational, novel therapies for our patients. I conclude by discussing present and future applications for various IL-2R-directed ap-
Table 3. Summary of In-Vitro Activities of Antibody Constructs

<table>
<thead>
<tr>
<th>Antibody Class</th>
<th>Antibody</th>
<th>Binding (K_M/M)</th>
<th>Block T-Cell Proliferation</th>
<th>Recruit Cellular Killing by Antibody-dependent Cellular Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Anti-Tac Mouse G2a</td>
<td>(9 \times 10^9)</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Chimeric</td>
<td>Human G1</td>
<td>(9 \times 10^8)</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Chimeric</td>
<td>Human G3</td>
<td>(9 \times 10^8)</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Hyperchimeric</td>
<td>Human G1</td>
<td>(3 \times 10^8)</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
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Approaches we are developing. As we have been discussing, one major goal is to use IL-2R-directed therapy to treat patients with Tac-expressing malignancy. A second clinical target with an even wider potential for use is the treatment of T-cell-mediated autoimmune disorders. I will now conclude by focusing on a third application that is close to realization, the use of IL-2R-directed therapy in the treatment of graft-versus-host disease and in the prevention of allograft rejection.

The rationale for using IL-2R-directed therapy to prevent allograft rejection is that the T cells of the host recognize the foreign transplantation antigens on the allograft, become activated, display the IL-2R \(\alpha\) protein (Tac), and participate in organ rejection. Antibodies to the IL-2R inhibited the proliferation of T cells activated by interaction with foreign histocompatibility antigens expressed on the donor organ and prevented the generation of cytotoxic T cells in allogeneic cell co-cultures (73). Further, the survival of cardiac and renal allografts was prolonged in rodent recipients treated with appropriate IL-2R-directed monoclonal antibodies (74). We have extended these observations by demonstrating that the administration of the anti-Tac monoclonal antibody leads to the prolongation of cardiac and renal allograft survival in cynomolgus monkeys (75). In light of these encouraging results, human recipients of cadaver donor renal allografts were treated with anti-IL-2R monoclonal antibodies as adjunctive immunotherapy. In a randomized, prospective trial, anti-Tac was added to standard immunosuppression in the treatment of one of two groups of 40 patients after renal transplantation (76). There was no increased toxicity associated with the administration of anti-Tac, however. There was a significant reduction of early rejection episodes after renal transplantation by anti-Tac administration. These efforts were limited by the fact that murine anti-Tac was immunogenic and elicited human anti-mouse antibodies in the patients. To circumvent this problem, humanized anti-Tac was evaluated in the cynomolgus cardiac allograft transplantation model. When compared to the murine version, humanized anti-Tac was markedly less immunogenic and manifested improved pharmacokinetics with prolonged in-vivo survival. Furthermore, cardiac allograft survival was prolonged in the animals treated with humanized anti-Tac when compared with those treated with the murine monoclonal antibody (75). Humanized anti-Tac will soon be evaluated for toxicity and efficacy in patients with malignancies that express the IL-2 receptor in patients with graft-versus-host disease and in those receiving organ allografts.

The fusion proteins involving truncated \textit{Pseudomonas} exotoxin and IL-2 or the variable region of the anti-Tac...
monoclonal antibody may also have a niche in allograft protocols. Further, the specificity and potency of these toxin fusion proteins as cytotoxic agents may be of value in treating graft-versus-host disease and in treating acute allograft rejection. However, the most effective IL-2R-directed approach used in primate allograft models has been radiometal-chelated anti-Tac. Specifically, yttrium-90, a beta-emitting isotope, was chelated to anti-Tac using the chelating agent IB4M discussed by Dr. Gansow. Yttrium-90-anti-Tac has been evaluated for efficacy and toxicity in a primate xenograft organ transplantation model (77). Rhesus monkeys receiving a xenograft of a cynomolgus monkey heart manifested a marked prolongation (with a mean graft survival in five treated animals of 40 days compared with 7 days in five control animals) of xenograft survival when treated with 90Y-labeled anti-Tac on days 2, 4, 7, and 11 after transplantation. No prolongation of xenograft survival was observed after the administration of unmodified anti-Tac, and only a modest increase in survival occurred following the administration of 90Y on an irrelevant monoclonal antibody. Similarly, a marked prolongation of cardiac allograft survival in the cynomolgus monkey was achieved by the administration of 90Y-labeled anti-Tac (78). On the basis of these encouraging results, a phase I trial of 90Y-anti-Tac was initiated in patients with IL-2R-expressing adult T-cell leukemia. At the doses used (5 and 10 mCi per patient), no toxicity was observed in five of the six patients studied; modest granulocytopenia and thrombocytopenia were observed in one patient. Five of these six patients received a sustained or complete remission after 90Y-labeled anti-Tac therapy that has been maintained for the 8 months of study.

In summary, activation of resting T-cells induces the synthesis of IL-2 as well as the expression of high-affinity cell surface receptors for this lymphokine. Failure of the production of either IL-2 or its receptor results in a failure of T-cell immune response. Two IL-2 binding peptides, a 55-kDa IL-2R α chain reactive with the anti-Tac monoclonal antibody, and a novel 70- to 75-kDa IL-2R β chain, have been identified. Cell lines expressing both chains manifest high-affinity IL-2 binding. In contrast to resting normal T-cells that do not express high-affinity IL-2Rs, the abnormal T-cells of patients with leukemia-lymphoma or with selective autoimmune disorders and of individuals rejecting allografts express IL-2 receptors. To exploit this difference in IL-2R expression, therapeutic trials have been initiated using unmodified anti-Tac in the treatment of patients with Tac-expressing leukemia and lymphoma as well as those receiving organ allografts. Further, IL-2R-directed toxin fusion proteins have been used to target the toxin to IL-2R-expressing malignant cells. In parallel studies, alpha- and beta-emitting radiometal chelates of anti-Tac have been targeted to such cells for use in radioimaging or radioimmunotherapy. Humanized anti-Tac molecules have been prepared by genetic engineering in which the mouse molecule is entirely human IgG except for the small antigen binding regions that are retained from the mouse antibody. The IgG humanized anti-Tac monoclonal manifests a new activity of antibody-dependent cellular cytotoxicity with human mononuclear cells that is absent in the parental mouse anti-Tac. Thus our present understanding of the IL-2-IL-2R system opens the possibility for more specific immune intervention strategies. The IL-2R may prove to be an extraordinarily useful therapeutic target. The clinical application of IL-2R-directed therapy represents a new perspective for the treatment of certain neoplastic diseases, autoimmune disorders, and graft-versus-host disease, and for the prevention of allograft rejection.

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Functional and phenotypic comparison of human T cell activation


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What really is the doctor’s condition? What gives meaning to his activities? Practicing all the arts of medicine? Giving an injection in this case or prescribing a medicine in that? To practice all the arts of medicine is not to practice the art of medicine. The medical profession merely provides a framework wherein the doctor finds continual opportunities to fulfill himself through the personal exercise of professional skill. The meaning of the doctor’s work lies in what he does beyond his purely medical duties; it is what he brings to his work as a personality, as a human being, which gives the doctor his peculiar role. Only when he goes beyond the limits of purely professional service, beyond the tricks of the trade does he begin that truly personal work which alone is fulfilling.

Viktor E. Frankl, MD
The Doctor and the Soul
Vintage Books, 1955, p. 119