Symposium article

T-cell receptors for cytokines: Targets for immunotherapy of leukemia/lymphoma*

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Summary

Background: Cytokine receptors are exceptionally valuable targets for immunotherapy. For example, the high affinity IL-2 receptor is expressed by abnormal T cells in patients with certain lymphoid malignancies or autoimmune disorders and in individuals rejecting allografts whereas it is not expressed by normal resting cells.

Design: To exploit this difference in receptor expression in normal resting cells and leukemic cells we have introduced different forms of IL-2 receptor directed therapy including an unmodified murine antibody to the α subunit of the IL-2 receptor (anti-Tac), humanized anti-Tac as well as this antibody armed with truncated Pseudomonas exotoxin or α- and β-emitting radionuclides (e.g., ²¹¹At and ⁹⁰Y). In particular, unmodified murine anti-Tac was used in the therapy of HTLV-I-associated adult T-cell leukemia (ATL).

Results: Six of nineteen patients treated with this antibody underwent a partial (four) or complete (two) remission. In a subsequent clinical trial involving anti-Tac armed with ⁹⁰Y over 50% of the patients with ATL treated underwent a partial or complete remission.

Conclusions: New agents under development include humanized antibodies directed toward shared cytokine receptors such as IL-2/15Rβ used by both IL-2 and IL-15 as well as to a shared signal transduction element Jak3 utilized by the T-cell stimulatory cytokines IL-2, IL-4, IL-7, IL-9 and IL-15. Thus our emerging understanding of cytokine receptors and their signaling pathways taken in conjunction with the ability to produce humanized antibodies armed with radionuclides or toxins are providing novel perspectives for the treatment of leukemia and lymphoma.

Key words: adult T-cell leukemia, anti-Tac mAb, cytokine, HTLV-I, interleukin-2, interleukin-15

The hybridoma technique of Köhler and Milstein rekindled interest in the use of antibodies as therapeutic agents in the treatment of patients with cancer [1]. Initially such monoclonal antibodies (mAbs) had only modest effects against tumor cells in clinical trials [2, 3]. However, as noted in Science 1998; 280: 1196, 'Antibodies stage a comeback in cancer treatment' [4] anti-bodies have received FDA approval [4]. At the present time, FDA approval has been achieved for Rituxan (anti-CD20), Herceptin® (anti-HER-2/neu), and Zenapax® (anti-IL-2Ra, CD25), among others [5-8]. A number of factors underlie the improved efficacy of mAb therapy for cancer. First of all human or humanized antibodies have been developed that manifest reduced immunogenicity, have augmented effector functions, and have markedly improved pharmacokinetics with prolonged in vivo survivals when compared to unmodified murine antibodies. A second critical advance is that more effective antigenic targets have been identified including growth factor and death pathway receptors. In the case of growth factor receptors cytokine deprivation, mediated apoptotic cell death of the leukemic cells can be induced by interdicting the interaction of the growth factor with its receptor. In particular, receptors for interleukins-2 and -7 have been targeted in monoclonal antibody therapy of leukemia and lymphoma [8-12]. A third advance involves the arming of antibodies with toxins or radionuclides thereby enhancing their effector functions.

We have readdressed this issue utilizing the IL-2 receptor alpha subunit (IL-2Ra) (CD25) identified by the anti-Tac mAb as a target for immune intervention [8-11, 13-15]. The scientific basis for this approach is that resting normal cells including the normal cells of patients with leukemia do not express IL-2Ra in contrast to select malignant T cells including those of patients with HTLV-I-associated ATL [15], and those with cutaneous T-cell lymphoma [17] that express this receptor. Furthermore, IL-2Ra is constitutively expressed by the malignant cells of hairy cell B-cell leukemia, and the Reed-Sternberg cells of Hodgkin's disease [18, 19].

To exploit the differences in IL-2R expression between normal and leukemic cells a number of IL-2R-directed approaches have been introduced including: (1) unmodified murine antibodies (Mu-anti-Tac) directed toward IL-2Ra, (2) humanized versions of the anti-Tac mono-

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clonal antibody, as well as (3) the anti-Tac mAb armed with toxins or radionuclides. Finally, receptors (e.g., IL-2/15Rβ) shared by multiple cytokines (e.g., IL-2 and IL-15) as well as molecules in the signaling pathway (e.g., Jak3) used by diverse T-cell stimulatory interleukins (IL-2, IL-4, IL-7, IL-9 and IL-15) are being targeted for therapy of leukemia/lymphoma.

**IL-2/IL-2R system and its signaling pathway**

IL-2 is a 15.5 kDa glycoprotein that exerts its effects on activated T cells by binding to the high-affinity form of the IL-2R. This high-affinity IL-2R is composed of three distinct membrane components: The 55 kDa IL-2Rα chain (Tac, CD25), the 70–75 kDa IL-2Rβ chain (CD122) and the 64 kDa γc chain (CD132) [8, 20]. Cytokines such as IL-2 manifest considerable redundancy that is explained by sharing of common receptor subunits among members of the cytokine receptor family. Each of these cytokines has its own ‘private’ receptor but IL-2 also shares two of its receptor subunits. In particular, the β chain (IL-2/15Rβ) is shared by IL-2 and IL-15 and the γc chain is shared by five cytokines that activate T cells [21–24]. The β and γc chains (but not the α chain) are members of a superfamily of cytokine receptors characterized by four conserved cysteines and the Trp-Ser-X-Trp-Ser motif. IL-2Rα subunit associates with IL-2/15Rβ and γc subunits to form a high-affinity IL-2R complex [25].

The IL-2R in T cells and natural killer (NK) cells, like most cytokine receptors, does not possess intrinsic protein tyrosine kinase domains. However, receptor stimulation evokes rapid tyrosine phosphorylation of intracellular proteins including the receptors themselves. The IL-2R binds Jak1 and Jak3, members of Janus kinase (Jak) family of protein tyrosine kinases [26–28]. Jak activation by IL-2 in turn leads to phosphorylation and nuclear translocation of STAT-3 and STAT-5, two members of the transcription factor family known as signal transducers and activators of transcription (STATs) [27, 28].

**IL-2R expression in leukemia/lymphoma**

We have used the IL-2R as a target for immune intervention. The scientific basis for the approach utilizing the IL-2Rα subunit as a target for immunotherapy is that resting normal cells do not express this subunit of the IL-2R. In contrast, this receptor is expressed by a proportion of the abnormal cells in certain forms of lymphoid neoplasia, select autoimmune diseases, and in individuals rejecting allografts [15, 16]. That is, a proportion of the abnormal cells in these diseases express the IL-2Rα on their surface. Furthermore, the serum concentration of the soluble form of IL-2Rα is elevated in patients with these disorders [29]. In terms of neoplasia, certain T-cell, B-cell, monocytic, and even granulocytic leukemias express IL-2Rα (CD25). Specifically, the malignant T cells of patients with human T-cell lymphotropic virus I (HTLV-I)-induced ATL constitutively express IL-2Rα [16]. Furthermore, the malignant T cells of the skin and lymph nodes of patients with cutaneous T-cell lymphoma (mycosis fungoides and the Sézary syndrome) express the Tac antigen [17]. In addition, virtually all of the malignant cells of patients with hairy cell B-cell leukemia and a proportion of other B-cell lymphomas are also Tac-positive [19]. Finally, true histiocytic leukemia cells and the Reed–Sternberg cell of Hodgkin’s disease also manifest IL-2Rα [18]. In addition to these Tac-expressing leukemias and lymphomas, there are certain leukemias (e.g., acute lymphoblastic leukemia and large granular lymphocytic leukemia) that do not express IL-2Rα, yet express the IL-2/15Rβ subunit of the IL-2 and IL-15 receptors [30].

**Disorders of IL-2R expression in HTLV-I-associated ATL**

We focused our initial IL-2R immunotherapeutic studies on ATL, a distinct form of aggressive T-cell leukemia [15, 31]. HTLV-I is the primary etiologic agent in ATL [32]. ATL is a malignant proliferation of CD3/CD4-expressing T cells that typically infiltrates the skin, lungs and liver. All populations of leukemic cells we have examined from patients with HTLV-I-associated ATL constitutively express high- and low-affinity IL-2R, including very large numbers of the IL-2Rα defined by the anti-Tac mAb [16]. An analysis of HTLV-I and its protein products suggests a potential mechanism for the association between HTLV-I and constitutive IL-2Rα expression [33]. The retrovirus HTLV-I encodes a 42 kDa protein (termed tax) that plays an important role in the early phases of HTLV-I-induced malignancy by deregulating the expression of the cellular genes that encode IL-2 and IL-2Rα thereby establishing an autocrine stimulation of proliferation that can be inhibited by anti-Tac ex vivo.

**IL-2Rα as a target for therapy in patients with HTLV-I-associated ATL**

*Unmodified anti-Tac monoclonal antibody*

No conventional treatment program is successful in inducing long-term disease-free survival in ATL patients. A total of 854 patients with HTLV-I antibody positive ATL newly diagnosed from 1983 to 1987 were analyzed for prognostic factors and survival following combined therapy by the lymphoma study group [34]. The median survival time and projected two- and four-year survival rates of all patients were 10 months, 28% and 12%, respectively. However, the HTLV-I-induced ATL cells constitutively express the IL-2Rα chain identified by the anti-Tac mAb whereas normal resting cells do not. This
observation provided the scientific basis for IL-2R-directed immunotherapy with this mAb. IL-2R-directed immunotherapeutic agents could theoretically eliminate IL-2Rα-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-mediated immune responses. In our initial studies we administered unmodified murine anti-Tac to patients with ATL [9]. Our goal was to inhibit the interaction of IL-2 with its growth factor receptor expressed on the malignant cells. Six of the nineteen treated patients had a partial (four patients), or complete (two patients) remission, lasting from one month to more than nine years after anti-Tac therapy [9]. 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-I proviral integration and T-cell antigen receptor gene rearrangements.

**Humanized antibodies to the IL-2Rα**

Although murine antibodies, such as murine anti-Tac, are of value in the therapy of human disease, their effectiveness is limited because rodent mAbs have a short in vivo survival in humans, induce an immune response that neutralizes their therapeutic effect, and as is the case with anti-Tac are ineffective at recruiting host effector functions. We addressed these issues by joining with Cary Queen in the development and evaluation of Hu-anti-Tac [35, 36]. The Hu-anti-Tac molecules retain the complementarity-determining regions from the murine antibody but have virtually all of the remainder of the molecule derived from human IgG1κ. Hu-anti-Tac had improved pharmacokinetics when compared with the murine version with an in vivo survival that was longer (terminal T½ 103 hours vs. 38 hours in cynomolgus monkeys and 20 days vs. 40 h in humans). In addition, Hu-anti-Tac was markedly less immunogenic than murine anti-Tac when administered to monkeys undergoing heterotopic cardiac allografting or to patients with leukemia/lymphoma or those receiving renal allografts [37, 38]. Furthermore, in contrast to the parent murine anti-Tac, Hu-anti-Tac participated in ADCC with human mononuclear cells [36].

**Trials involving Hu-Anti-Tac (Zenapax®) in benign conditions and leukemia**

On the basis of encouraging observations in preclinical trials and in phase I–II trials of Hu-Anti-Tac, two double blind (placebo controlled) randomized trials involving 535 evaluated patients were conducted to determine the value of Hu-Anti-Tac (Zenapax®) in preventing renal allograft transplant rejection [7]. In each trial the patients received a standard immunosuppressive agent regimen (cyclosporin A and hydrocortisone in one study; cyclosporin A, hydrocortisone and azathioprine in the other). The parallel treatment groups received either an intravenous placebo or a dose of 1.0 mg/kg of Zenapax® prior to transplant and on four subsequent occasions separated by two weeks. No drug-specific adverse events or increased morbidity were observed. In particular, there was no increase in the incidence of infections or of B-cell lymphoma in the group receiving Zenapax®. Acute rejection episodes were reduced by 40% in patients treated with Zenapax® (P < 0.001). Ninety-eight percent of the patients receiving triple immunotherapy and Zenapax® retained their renal allograft for six months whereas only ninety-two percent of the patients in the placebo control group retained their grafts (P < 0.02). On the basis of these phase III clinical trials the Food and Drug Administration (FDA) on 10 December 1997, approved Zenapax® for use in humans (marketing clearance) to prevent acute kidney transplant rejection [8]. In addition to its use in the prevention of organ allograft rejection, Zenapax® (Hu-anti-Tac) appears to be of value in the therapy of T-cell mediated autoimmune disorders such as T-cell mediated uveitis and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a neurological disorder that results from HTLV-I infection leading to immune activation [39, 40]. In this disorder as in ATL HTLV-I encoded Tax transactivates the expression of IL-2 and the IL-2 receptor. Anti-Tac blocks the interaction of IL-2 with IL-2Rα. Finally humanized anti-Tac therapy has been associated with remissions in patients with ATL, especially in patients with smoldering and chronic forms of the disease [Waldmann, unpublished observation].

**Monoclonal antibody/cytotoxic agent conjugates**

During the progression of ATL the malignant cells continue to express IL-2Rα but no longer produce or require IL-2 for their proliferation and survival. Nevertheless, the signalling pathway involving Jak1 and Jak3 as well as STAT5 remains activated. A number of factors underlie this IL-2 independent activation. HTLV-I tax transactivates IL-15 which acts on the private IL-15Rα and on IL-2/15Rβ and γc shared with IL-2 [41]. In addition, in IL-2 independent ATL cell lines there is a loss of SHP-1, the phosphatase that normally inactivates Jak3 [42]. Nevertheless, IL-2Rα is still expressed on the leukemic cells but not on the normal cells of the patients, thus providing a target for immunotherapy.

**IL-2R-directed immunotoxins**

The limited efficacy of unmodified mAbs in the therapy of the late phase of ATL led us to an alternative
approach, the use of anti-Tac as a carrier of cytotoxic substances including toxins or α- and β-emitting radionuclides. In one group of studies, we collaborated with Ira Pastan and Robert Kreitman (LMB, NCI) in the study of IL-2Rα-directed immunotoxins involving a version of *Pseudomonas* exotoxin (PE38) that has a deletion of domain I, the domain responsible for unwanted ubiquitous binding. A single chain toxin fusion protein, anti-Tac Fv-PE38, in which the variable region (Fv) of anti-Tac was joined in peptide linkage to PE38 had the characteristics *in vitro* and *in vivo* with IL-2Rα-expressing tumor models in mice that suggested its use as an IL-2R-directed agent in humans [43]. Therefore in collaboration with Pastan and Kreitman, a phase I trial of the immunotoxin anti-Tac Fv-PE38 has been completed that evaluated the immunogenicity, toxicity and efficacy of this onco toxin in the treatment of patients with a wide variety of IL-2Rα-expressing leukemias and lymphomas including hairy cell leukemia and ATL [44]. Of the 35 patients treated one HCL patient had a complete remission. Furthermore, there were seven partial responses (HCL) (3), chronic lymphocytic leukemia (1), Hodgkin’s disease (1), CTCL (1), and ATL (1). All five patients with HCL or CTCL and one of two patients with ATL responded.

**Anti-Tac armed with α- and β-emitting radionuclides**

The action of toxins conjugated to mAbs depends on their ability to be internalized by the cell and translocated to the cytoplasm. In fact, the toxin conjugates do not pass easily from the endosome to the cytosol. Furthermore, large protein toxins are immunogenic and thus provide only a narrow therapeutic window prior to the development of host antibodies directed toward the toxin. In the future these problems will probably be resolved. However, to circumvent these limitations radiolabeled mAbs were developed as alternative immunoconjugates to deliver a cytotoxic agent to target cells. There are a number of advantages over other approaches in the use of radiolabeled mAbs conjugates for therapy. One is that with the appropriate choice of radionuclide, radiolabeled mAbs kill cells at distances of several cell diameters; therefore, a radiolabeled mAb binding to an antigen-expressing cell may kill adjacent antigen-non-expressing cells, thereby overcoming the tumor cell antigenic heterogeneity that presents a problem for most other mAb-mediated approaches. Furthermore, the radiolabeled antibody need not be internalized to kill the tumor cell. Nuclear chemistry has provided a selection of radioisotopes that could be linked to immuno-proteins.

In studies performed in collaboration with O. Gansow and M. Brechbield, we turned to α- and β-emitting radionuclides as cytotoxic agents that could be conjugated to anti-Tac [45, 46]. In initial studies a series of linker agents was developed that did not compromise antibody specificity and did not permit premature release of the radionuclide *in vivo* [45]. Our choice of isotopes is based on the desire to have agents with a short distance of action that would act on the cell in question and on a small number of bystander cells without unwanted toxicity.

Radionuclides emitting α particles release high-energy emission (6–9 MeV) over a short distance (40–80 μm) and are efficient at killing individual target cells such as those found in patients with leukemia without significantly penetrating normal tissues. Suitable emitting radionuclides that are under investigation include 213Bi, 212Bi, 210Pb, and 211At. We have shown with *in vitro* studies that 212Bi was well suited for immunotherapy [47]. Activity levels of 0.5 μCi targeted by 212Bi-labeled anti-Tac eliminated >98% of the proliferative capacity of HuT-102 cells, with only a modest effect on IL-2R-negative cells. In addition, an *in vivo* tumor model in nude mice was used to show that 212Bi-labeled anti-Tac used at a dose that produced only modest toxicity was effective in preventing the development of tumors expressing the Tac antigen [48]. 211At with its longer physical T2 7.2 hours vs. 1 hour for 212Bi and its almost pure α-emission appears to be even more suitable for therapy. Therefore we plan to initiate the clinical trials of 211At-labeled anti-IL-2Rα mAb for the treatment of IL-2R-expressing leukemia/lymphoma.

The focus of our program involving IL-2R-directed mAbs armed with radionuclides has been on the use of 90Y linked to anti-Tac [10]. We administered anti-Tac armed with 90Y to 18 patients with ATL, initially (the first nine patients) in a phase I dose-escalation trial and subsequently (the second group of nine patients) in a phase II trial involving a uniform 10 mCi dose of 90Y-labeled anti-Tac. Patients undergoing a remission were permitted to receive up to eight additional doses [10]. Nine of the sixteen evaluable patients manifested partial (7) or complete (2) remissions induced by courses of 90Y anti-Tac therapy. The duration of the seven partial remissions ranged form 1.6–22.4 months (mean 9.2 months). Two additional patients developed a complete remission. The observations that support these conclusions concerning the favorable therapeutic responses include the demonstration of a reduction in size of all measurable lesions as assessed by physical examination, computed tomography scan, and γ camera imaging studies after intravenous coadministration of 111In-anti-Tac. Furthermore, the clinical responses in all patients with leukemia were associated with a reduction in the number of peripheral blood leukemia cells enumerated by fluorescence-activated cell sorting analysis, by a decline in the serum IL-2Rα concentration, and by a normalization of the Southern blot patterns of T-cell receptor β gene arrangement and HTLV-I integration. The responses observed represent an improved efficacy in terms of length of remission compared with previous results with unmodified anti-Tac. Clinically meaningful (> grade 3) toxicity was limited largely to the hematopoietic system.
Future directions

Although IL-2Rα-directed therapy has met with considerable success there are limitations in approaches directed solely to this receptor subunit. Antibodies to IL-2Rα do not inhibit IL-2-mediated activation of NK cells which in their resting state do not express IL-2Rα but do express IL-2/IL-15Rβ and γc [49]. Furthermore, antibodies to IL-2Rα do not inhibit the action of IL-15 that utilizes its own private receptor (IL-15Rα) rather than IL-2Rα [50]. Finally, anti-IL-2Rα antibodies alone do not provide the virtually complete immunosuppression of T cell function that could be achieved if the actions of all the cytokines that stimulate T cells were inhibited. These limitations are relevant to leukemia therapy since certain leukemias such as T-cell type large granular lymphocyte (LGL) express IL-2/IL-15Rβ and γc but not IL-2Rα.

As noted above, there is a sharing of receptor subunits and signaling pathway elements between IL-2 and its receptor plus those of the other cytokines that stimulate T cells. A major corollary of this sharing of cytokine receptor subunits and signaling pathways among the cytokines is that therapy directed toward a shared cytokine receptor (e.g., IL-2/IL-15Rβ or γc) or to a shared signal transduction element (e.g., Jak3) may yield more profound immunosuppression than can be achieved by an antibody directed toward a private receptor subunit such as IL-2Rα. Tinubu et al. [51] demonstrated that a humanized version of the Mikβ1 antibody directed toward the IL-2/IL-15Rβ that is shared by IL-2 and IL-15 prolongs renal allograft survival in cynomolgus monkeys. Furthermore, we are evaluating the value of Mikβ1 in the therapy of patients with T-cell type LGL associated with hematocytopenia.

Many groups have initiated programs directed toward developing and evaluating inhibitors of Jak3 as agents for controlled immunosuppression. Jak3 is involved in the signaling of some cytokines including IL-2 and IL-15 that employ γc but is not essential for signaling by other growth factors. Jak3 expression is largely limited to lymphocytes and hematopoietic cells. Jak3 deficiency in the autosomal form of severe combined immunodeficiency disease (Jak3-deficient SCID) in humans yields immunodeficiency but no disorders in nonimmunological systems [52,53]. In parallel, mice made Jak3-deficient by gene-targeting manifested an absence of NK cells and abnormalities of T- and B-cells but, like the Jak3-deficient humans, do not have disorders in nonimmunological systems [54]. Taken together, these observations suggest that agents that inhibit Jak3 action may be of value as therapeutic agents in patients with leukemia/lymphoma. In conclusion, the emerging understanding of the IL-2/IL-15R system opens the possibility for more specific immune intervention. This understanding taken in conjunction with the ability to produce humanized antibodies to the IL-2R subunits by genetic engineering and to arm these antibodies with toxins or with α- or β-emitting radionuclides may provide a rational therapeutic strategy for the treatment of IL-2R-expressing leukemias and lymphomas including HTLV-I-associated ATL.

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