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Cytokines with Possible Clinical Utility

R. Mertelsmann [1], J. Kolitz [2], K. Welte [2], and F. Herrmann [1]

A. Introduction

Biological response modifiers (BRM) are agents aimed at reducing tumor growth, not primarily by exerting direct cytotoxic effects but by modulation of tumor gene expression (e.g., induction of differentiation) or by enhancing host defense mechanisms directed against cancer cells. BRM as primary therapy or as adjuncts to cytotoxic agents in the treatment of cancers have attracted increasing interest in view of stagnating clinical results in many areas [1], and there is increasing evidence of in vitro and in vivo efficacy of these agents. Furthermore, advances in molecular biology suggesting that oncogenes and their products play a crucial role in oncogenesis support approaches to modulation of regulatory mechanisms as a means of controlling tumor cell growth.

[1] Department of Hematology, University of Mainz, Mainz, Federal Republic of Germany
[2] Laboratory of Cytokine Biology, Sloan-Kettering Institute, New York, NY, USA

Clinical trials of BRM are more complex than those evaluating cytotoxic agents which are generally given at maximum tolerated dosages. Maximum tolerated doses of BRM are not necessarily optimal for modifying biological response, nor are they always the most efficacious doses. A tentative classification for BRM with some representative agents is presented in Table 1.

Agents such as retinoic acid affect tumor cell proliferation and differentiation, apparently through modulation of tumor cell gene expression rather than through host mechanisms. It should be kept in mind, however, that tumor-host interactions are subject to an intricate regulatory network of cells and cytokines, similar to the endocrine system. Modulation of one parameter could have additional, indirect effects on the biological response network. Table 2 describes cytokines that have been cloned and have thus been identified as unique gene products.

It has now become increasingly clear that the original hypothesis of "one producer cell type – one cytokine – one target cell type" does not reflect the biological facts. Ample

Table 1. Biological response modifiers

Group	Examples
1. Monoclonal antibodies	Anti-melanoma, anti-T cell
2. Cytokines	IL1, 2, 3; IFN α, β, γ; TNF α, β; G-, g/M0-, M0-CSF, EPO, EPA
3. Synthetic agents	retinoid acid, vitamin D3, HMBA
4. Immunoregulatory peptides	Tuftsin, endorphins

IL1, 2, 3; Interleukin 1, 2, 3; *IFN* α, β, γ, interferon α, β, γ; *TNF* α, β, tumor necrosis factor α, β; *CSF for G, G/M0, M,* colony stimulating factors for granulocytes, granulocytes/macrophages, macrophages; *EPO,* erythropoietin; *EPA,* erythroid potentiating activity; *HMBA,* hexamethylene bisacetamide

Table 2. Recombinant human cytokines

Agent	Receptor	Hemopoietic producer cell	cDNA cloning reported by	Growth factor for	Activation factor for
IL1	–[a]	M0	March et al. (1985) Nature 315:641	T, B	T, B
IL2	Tac	T	Taniguchi et al. (1983) Nature 302:305	T, NK, B, M0[c]	T[c], NK[c], B, M0[c]
IL3	–	T	Not published	G/M0-PC, early RBC-PC	nd[d]
IFNα	–	B, M0, Nk	Goeddel et al. (1980) Nature 287:411	–	NK[c]
IFNβ	–	–	Derynck et al. (1980) Nature 285:542	–	–
IFNγ	–	T, NK	Gray et al. (1981) Nature 295:503	–	NK, M0[c]
TNFα	–	M0	Shirai et al. (1985) Nature 312:803	T (?)	M0[c]
TNFβ	–	T	Gray et al. (1984) Nature 312:721	nd	T
G-CSF	–	M0	Souza et al. (1986) Science 232:61	G-PC	G
GM-CSF	fes (?)[e]	T	Wong et al. (1985) Science 228:810	G/M0-PC, early RBC-PC	M0
M-CSF	fms	M0	Kawasaki et al. (1985) Science 230:291	–	M0
EPO	–	–	Lin et al. (1985) Proc Natl Acad Sci USA 82:7580	late RBC-PC[c]	–
EPA	–	T	Gasson et al. (1985) Nature 315:768	early and late RBC-PC	–

[a] Not cDNA cloned. *MO,* macrophages; *T,* T cells; *B,* B cells; *NK,* NK cells; *G,* granulocytes; *RBC,* red blood cells; *PC,* progenitor cells.
[b] Documented in clinical trials.
[c] Not determined.
[d] Related oncogene.

evidence has been accummulated demonstrating that a given cytokine can be produced by different cell types (e.g., IL1) and can exert effects on different cell types (e.g., IL2 on T cells, B cells, and monocytes). The biological effect appears to be dependent upon receptor density, receptor affinity, and ligand concentration, as well as on the functional state of the responder cell [2]. Since so far only one of these agents, IL2, has undergone more than preliminary clinical evaluation, the following brief review will focus on this agent.

B. Interleukin 2

The long-term proliferation of normal T-lymphocytes in suspension culture was first achieved by Morgan et al. [3], using the T-cell-derived cytokine intially designated "T-cell growth factor" (TCGF) and later renamed interleukin 2 (IL2).

The biochemical purification of IL2 and the molecular cloning and expression of its gene have led to a growing appreciation of the protean functional capabilities of this molecule. It supports the growth of human cytotoxic T cells (CTL) [4, 5] and natural killer (NK) cells [6], it enhances the functional capabilities of NK cells [1, 8], and it is the factor essential for the induction and growth of human lymphokine-activated killer (LAK) cells [9]. It induces antigen-specific T-cell lines to produce B-cell growth factor-I [10], and it is capable of enhancing gamma-interferon (gamma-IFN) production either alone [11] or in conjunction with mitogen [2]. An even broader immunoregulatory role for IL2 is suggested by its recently demonstrated ability to drive B cell proliferation and immunoglobulin production (13–16) and by the recent description of functionally active IL2 receptors on macrophages [12].

Not surprisingly, a number of human disease states have been found to be associated with varying defects in IL2 production and response. Among those affected are patients with primary and acquired immunodeficiency diseases, including common variable immunodeficiency (CVI) [18] and the acquired immunodeficiency syndrome (AIDS) [19, 20], bone marrow transplant recipients [21], and patients with severe burns and hemophilia [22] (K. Welte, unpublished observations). Furthermore, several immunosuppressive drugs appear to exert their effects by blocking IL2 gene expression [23, 24]. Defects in lectin- and mitogen-induced T-cell proliferation are frequently reversible in vitro by exogenous IL2. These observations, coupled with the demonstrated ability of IL2 to enhance the cytotoxicity of NK and LAK cells, have provided a rationale for clinical evaluation of IL2 in human malignancy and immunodeficiency.

The human IL2 gene has been cloned and sequenced [25], and its position on chromosome 4 has been determined by us and others [26, 27]. Several recombinant IL2 (rIL2) preparations (Cetus, Amgen, Biogen) have been compared in our laboratory with human purified IL2 (hpIL2) and, except for higher background mitogenic activity on the part of rIL2, no differences were detected in a variety of human in vitro and murine in vitro and in vivo systems (K. Welte, V. J. Merluzzi, unpublished observations).

C. IL2 in the Treatment of Cancer

The ability of IL2 to restore T cell functional defects in vivo and in vitro and to induce and enhance cytoxicity against fresh and cultured tumor targets led to early exploration of its potential as an agent in the treatment of cancer. The anti-tumor activity of IL2 has been most clearly demonstrated in conjunction with the infusion of specific immune cultured T cells or nonspecific LAK cells.

B6 mice with syngeneic Friend virus-induced FBL-3 leukemia are cured with the combination of noncurative doses of cyclophosphamide and administration of tumor-immune congeneic lymphocytes cultured in vitro and expanded in vivo with IL2 [28]. High doses of IL2 and infusions of autologous LAK cells cause major regressions of murine transplantable sarcomas and melanomas [29], with IL2 inducing in vivo proliferation of the adoptively transferred cells [30]. IL2 alone causes major regressions of murine sarcomas when given in extremely high doses (400 000 U intraperitoneally every 8 h) [31].

Following i.v. bolus administration of Jurkat hpIL2, the serum half-life of IL2 in

man was 5–7 min, with a second component of clearance of 30–120 min [32]. Such a two-compartment model is compatible with our own observations following treatment of 30 patients with rIL2 (Cetus) given by 6 hour continuous i.v. infusion [35].

We completed an initial trial of hpIL2 in human malignancy and immunodeficiency at Memorial Hospital in 1983 [33]. The IL2 was purified from human PBL-conditioned medium in our laboratory [34]. The s.c. route of administration was chosen in order to achieve maximal lymphatic drainage. Escalating doses were given, to a maximum daily dose of 20000 U/m^2 and a maximum total dose of 855000 U/m^2, administered over 77 days.

Sixteen patients with malignancy and AIDS were treated. Except for occasional skin irritation at the injection site, no toxicity was observed. One patient, a child with probable Nezelof's syndrome who died of infectious complications after 5 days of therapy with IL2 and after an unsuccessful T-cell-depleted bone marrow graft from a haplotype-identical half brother, was found at autopsy to have all lymph nodes lymphocyte depleted, except for inguinal nodes proximal to s.c. IL2 injection sites, where lymphoid follicles were noted. This was an early suggestion of the in vivo biological activity of IL2.

While there was some suggestion of improved responsiveness to OKT3-inducible T-cell activation in the only two patients receiving treatment for at least 50 days, there was no clear evidence for significant biological response modification in this trial.

A trial of Jurkat hpIL2 in human malignancy has been completed at the National Cancer Institute [32]. Twelve patients with a range of solid tumors received IL2 at doses of up to 2000 μg by i.v. bolus or continuous infusion weekly for 4 weeks. Biological observations included an acute decrease in peripheral blood T cells, affecting all major T cell subsets, and an increase in circulating cells capable of responding to IL2 and expressing LAK activity. No clinical response was seen. Toxicity consisted primarily of fever, chills, malaise, and reversible hepatopathy.

In a recently completed clinical trial, we administered rIL2 (Cetus) as a continuous 6-h i.v. infusion to 17 patients with advanced malignancy and to 13 patients with AIDS [35]. The maximum tolerated dose was 1000000 U/m^2, with dose-limiting toxicity consisting of fever >40 °C, thrombocytopenia, and diarrhea at the 2000000 U/m^2 dose level. Except for one patient with a myelodysplastic syndrome, who had a fall in marrow blasts from >10% to 1% over a 2-month period, no significant clinical responses were seen. Dose-dependent biological response-modifying effects were observed, however.

At the higher dose levels a reproducible lymphocytosis occurred, peaking on day 15 of each treatment cycle, with an up to five-fold increase in the absolute lymphocyte count. The expansion consisted of a polyclonal increase in all T-cell subsets, with no substantive change observed in any T-cell marker or in the T4/T8 ratio.

Twenty patients with solid tumors were treated with rIL2 (Cetus) at the National Cancer Institute [36] using i.v. bolus administration. No clinical responses were seen, but a Tac+ lymphocytosis was also observed, along with induction of detectable gamma-interferon serum levels.

Much interest has recently been generated by the report of major tumor regressions in patients with solid tumors (primarily melanoma, colon carcinoma, and hypernephroma) treated with infusions of autologous LAK cells and high doses (100000 U/kg every 8 h i.v.) of rIL2 [37]. A major focus of research activity will be to reduce the considerable toxicity of this approach, which has included marked fluid retention, pulmonary edema, hypotension, and reversible renal dysfunction. Whether the therapeutic effect is due primarily to the infusions of LAK cells or to the high doses of rIL2 is also presently unclear.

Given the ability of IL2 to induce LAK cells with wide anti-tumor efficacy both in vivo and in vitro and the clear demonstration of potent biological effects achievable in treated patients, additional efforts will have to be made to translate the promise of this lymphokine into clinically meaningful results. Subcutaneous, i.p., (P. Chapman et al., submitted) and intralesional administration might achieve sufficiently high local concentration of IL2 to generate LAK cells in vivo

with acceptable toxicity. The use of cyclophosphamide in low doses directed against suppressor T cells is a potential means of countering regulatory mechanisms limiting the efficacy of IL2 (J. Kolitz, manuscript in preparation). Defining the phenotype and optimizing the activation conditions for LAK cells may lead to therapies with reduced toxicities. The use of monoclonal antibodies directed against tumor antigens might lead to local inflammatory infiltrates in tumor sites [38]. CTL numbers and NK/LAK cytotoxicity could then possibly be amplified in vivo by IL2. These approaches are being utilized in current or planned clinical trials at Memorial Hospital, New York, and at the Department of Hematology of the University of Mainz.

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