Interleukin 2 Regulates Expression of Its Receptor and Synthesis of Gamma Interferon by Human T Lymphocytes

Abstract. *Interleukin 2 (IL-2) has an important role in the regulation of the expression of IL-2 receptors and the synthesis of gamma interferon (IFN-γ) by T lymphocytes. IL-2 is required for the optimum expression of IL-2 receptors on activated T lymphocytes and for maximum synthesis of IFN-γ in vitro. Dexamethasone, an immunosuppressant drug that inhibits IL-2 synthesis, diminished the expression of IL-2 receptors and the synthesis of IFN-γ. Anti-Tac, a monoclonal antibody known to prevent the binding of IL-2 to its receptor without inhibiting IL-2 synthesis, down-regulated the expression of the receptor and partially inhibited synthesis of IFN-γ. In a population of T lymphocytes prevented from synthesizing IL-2 by dexamethasone and incapable of using IL-2 as a result of blockage of IL-2 receptors by anti-Tac, the number of receptor-bearing cells and receptor density were diminished. Anti-Tac in combination with dexamethasone also exerted a synergistic effect on IFN-γ synthesis, inhibiting it almost completely. The inhibitory effect of dexamethasone IFN-γ synthesis may be of clinical importance, since IFN-γ activates macrophages and thereby triggers one of the defense mechanisms against bacterial infections.*

Earlier studies conducted in our laboratory have provided evidence that expression of interleukin 2 (IL-2) receptors could be induced on thymocytes by agents that induce the synthesis of gamma interferon (IFN-γ) (*1*). We have also shown that cyclosporin A, an immunosuppressant agent, inhibits synthesis of IFN-γ by thymocytes and T lymphocytes and synthesis of IL-2 by thymocytes (*2*). Other investigators reported that cyclosporin A inhibits the synthesis of IL-2 by T cells (*3*). IL-2 has been reported to augment IFN-γ synthesis by human thymocytes and mononuclear cells and to induce synthesis by unstimulated T cells (*1, 4*). In view of the importance of the function of IL-2 and IFN-γ in mounting an immune response, we investigated the role of IL-2 on the expression of IL-2 receptors and synthesis of IFN-γ. The effect of dexamethasone (Dex) on IFN-γ synthesis has not, to our knowledge, been reported. Anti-Tac, a monoclonal antibody that binds at or near the binding site of IL-2, has been found to block the binding of IL-2 and to down-regulate the expression of IL-2 receptors (*5*). Its effect on IFN-γ synthesis is not known. Anti-Tac does not inhibit IL-2 synthesis (*6*). The tumor promoter phorbol myristate acetate (PMA) exerts a synergistic effect with lectins on proliferation of thymocytes and T cells and on synthesis of IL-2 and IFN-γ. Synthesis of both IL-2 and IFN-γ precedes the mitogenic effect of the inducing agents (*1, 2*).

To study the relation between IL-2, the expression of IL-2 receptors, and synthesis of IFN-γ, we isolated mononuclear cells from peripheral blood by density centrifugation on a Ficoll-Hypaque gradient and removed macrophages by incubation on a plastic surface for 60 minutes. The cells were then passed through a nylon wool column and nonadherent cells (T cells) were washed and incubated (6×10^6 cells per milliliter) in complete RPMI 1640 medium (bovine calf serum, 10 percent; glutamine, 2m*M*; penicillin, 100 U/ml; streptomycin, 100 μg/ml; Amphotericin B, 0.25 μg/ml) or in complete medium containing PMA (1 ng/ml) for 3 hours at 37°C in a humidified atmosphere containing 5 percent CO_2. Cells were plated into flat-bottomed microtiter plates (96 wells, 200 μl) at a density of 6×10^6 cells per milliliter and cultured with agents that induce IL-2 synthesis [concanavalin A (Con A)] or with agents that either inhibit IL-2 synthesis (Dex) or inhibit IL-2 from binding to its receptors (anti-Tac). Cells were harvested and tested for viability and proliferative activity (data not shown). The expression of IL-2 receptors was determined by staining with immunoperoxidase as described (*1*). The concentration of IFN-γ was determined by a radioimmunoassay with two monoclonal antibodies to IFN-γ (*7*). An IFN-γ standard was included in each assay.

We found evidence that IL-2 modulates the expression of IL-2 receptors and IFN-γ production. IL-2 enhanced the percentage of receptor-bearing cells of activated T cells and increased IFN-γ synthesis. Conversely, T cells deprived of IL-2 by the combined effects of anti-Tac and Dex expressed IL-2 receptors only on a small percentage of cells, and their production of IFN-γ was inhibited.

T cells cultured with either Con A or PMA were induced to express IL-2 receptors (Table 1). The addition of exogenous IL-2 resulted in a threefold increase in the number of receptor-bearing cells. This increase was already detectable after 24 hours, before an increase in the proliferative rate could be detected (data not shown). The number of receptor-bearing cells could be increased further by incubating T cells with PMA and then adding Con A to cultures. PMA in combination with Con A produced a synergistic effect on the expression of receptors. IL-2 added to cultures of cells treated with PMA and Con A further increased the percentage of receptor-bearing cells, and most of the cells in culture were induced to express receptors for IL-2. The addition of IFN-γ to cultures of activated T cells had no significant effect on the expression of IL-2 receptors, indicating that the promotion

Table 1. Relation between IL-2, the expression of Tac^+ cells, and IFN-γ synthesis. Interleukin 2 (100 U/ml), Con A (10 μg/ml), Dex (10^{-6}*M*), and anti-Tac ascites (diluted to a final concentration of 10^{-4}) were added as indicated. (The IL-2 concentration used results in maximum stimulation of T cell proliferation under these conditions.) Cultures were incubated for 3 days. Tac^+ cells were determined with the monoclonal antibody anti-Tac and peroxidase-labeled goat antibody to mouse immunoglobulin G (BioRad Laboratories, Richmond, California). The IFN-γ titers were measured by a solid-phase radioimmunossay. An IFN-γ standard was included in each assay.

Additions	Tac^+ cells (%)		IFN-γ (U/ml)	
	Without IL-2	With IL-2	Without IL-2	With IL-2
Complete RPMI 1640 medium				
None	1	6	9	55
Con A	10	30	479	640
Con A + Dex	2	19	62	518
Con A + anti-Tac	8	28	128	324
Con A + Dex + anti-Tac	1	15	18	262
*Complete medium + PMA**				
None	2	9	108	1018
Con A	46	82	4070	5076
Con A + Dex	44	86	1364	1777
Con A + anti-Tac	48	43	3022	3888
Con A + Dex + anti-Tac	16	31	951	1036

*PMA (phorbol myristate acetate) at 1 ng/ml.

of the expression of IL-2 receptors was not mediated by IFN-γ (data not shown).

Dex and anti-Tac reduced the number of receptor-bearing cells in cultures supplemented with Con A alone. T cells treated with PMA and then cultured with Con A were more resistant to the inhibitory effect of Dex on the expression of IL-2 receptors. This inhibitory effect on cells activated with lectin could be reversed by exogenous IL-2. IL-2 also increased the number of receptor-bearing cells in cultures supplemented with Dex and induced with PMA and Con A. The combined effect of Dex (inhibition of IL-2 synthesis) and anti-Tac (blockage of IL-2 receptors) was synergistic. The expression of receptors induced with Con A alone or in combination with PMA was almost completely abrogated by anti-Tac in combination with Dex. Exogenous IL-2 had only a moderate effect in preventing this inhibition.

Synthesis of IFN-γ was modulated by IL-2 in a manner resembling the effect of IL-2 on its receptors. IL-2 served as an amplifying signal by augmenting IFN-γ synthesis. T cells cultured in complete medium produced low amounts of IFN-γ in culture, and exogenous IL-2 moderately increased their production of IFN-γ. This low degree of synthesis was increased by PMA and Con A. Maximum synthesis of IFN-γ was observed in cultures induced with Con A and PMA to which IL-2 was added. Maximum inhibition was achieved by the combined effects of Dex and anti-Tac. Dex inhibited synthesis of IFN-γ by T cells induced with Con A alone or in combination with PMA. This inhibition was readily overcome by IL-2 in cultures supplemented with Con A and was partially reversed in cultures containing PMA and Con A. Anti-Tac inhibited IFN-γ synthesis more effectively in T cells activated with Con A than in T cells cultured with PMA and Con A. Once the expression of IL-2 receptors was blocked by anti-Tac, IL-2 was not effective in reversing the inhibitory effect.

In conclusion, IL-2 has an important role in augmenting the expression of IL-2 receptors and in the synthesis of IFN-γ activated by T cells. In cultures of T cells whose supply of IL-2 has been cut off by Dex and anti-Tac, the number of cells displaying receptors and the density of receptors are decreased, and synthesis of IFN-γ ceases. This regulatory effect of IL-2 precedes T cell proliferation. The observation that Dex, an effective immunosuppressant drug, interferes with IFN-γ synthesis may be of clinical significance. Patients treated with glucocorticoids such as Dex risk infection partly because Dex inhibits the bacteriocidal effect of macrophages. Since IFN-γ is an activator of macrophages, these patients could be protected from infections by administration of IFN-γ.

Note added in proof: A similar observation that IL-2 was required for the optimal expression of IL-2 receptors on activated T cells has been made (*8*).

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Intestinal Uptake and Metabolism of Auranofin, A New Oral Gold-Based Antiarthritis Drug

Abstract. *Auranofin, 2,3,4,6-tetra-*O*-acetyl-1-thio-*β*-*D*-glucopyranosato-*S*-(triethylphosphine)-gold(I), an experimental antiarthritis pharmaceutical, metabolized in contact with hamster or rat gut wall to yield the deacetylated form of the drug. This product, 1-thio-*β*-*D*-glucopyranosato-*S*-(triethylphosphine)gold(I), passed through hamster or rat intestinal wall in an everted gut experiment. The metabolite was separated by high-performance liquid chromatography and characterized by retention time, chemical reactivity to yield a known product, and comparison to a synthetic sample of the metabolite.*

There is considerable evidence that auranofin, currently awaiting approval by the Food and Drug Administration for use in the United States (*1*), is effective in inducing remission of rheumatoid arthritis (*2*). However, little is known about the site of uptake of the drug, which is given orally, or the nature of the compounds circulating in patients after its administration. We used the everted sac model of intestinal absorption (*3*) to study uptake of auranofin. When everted intestinal sacs were incubated with a solution of auranofin for 2 hours, the gold concentration inside the sacs was about 20 percent of that in the incubation medium. Exposure to the intestinal tissue resulted in metabolism of auranofin. The product that accumulated inside the sac was the deacetylated form of the drug.

The everted sac preparation involves dissecting the intestine into segments, turning each segment inside-out, and tying it into a sac (*3*). The absorptive (mucosal) surface is thus exposed to the incubation medium, and the material that accumulates inside the sac has passed through the intestinal wall. For our experiments we used Syrian golden hamsters (150 g) and Sprague-Dawley rats (strain F344; 150 to 200 g). The intestine removed from an anesthetized animal was divided into two jejunum and two ileum segments. Segments were everted over polyethylene tubing, filled with Krebs-Ringer solution with bicarbonate buffer and 1 mg of glucose per milliliter, and tied into sacs. Sacs were incubated in this medium with auranofin for 2 hours at 37°C in a shaker moving at 1.33 Hz. The atmosphere was flushed continuously with 95 percent O_2 and 5 percent CO_2.

Both the incubation medium and the material collected from inside the sacs were assayed for glucose with Sigma kit 510 and for gold with inductively coupled plasma atomic emission spectroscopy (*4*). Since only those preparations in which glucose accumulates in the sacs against the concentration gradient maintain functional tissue, only those were analyzed further (typically nine of ten preparations). In these experiments the concentration of gold in the incubation medium ranged from 40 to 80 ppm. After 2 hours the concentration of gold in the sacs ranged from 15 to 30 percent of