IL-10 Synergizes with Dexamethasone in Inhibiting Human T Cell Proliferation

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ABSTRACT

We have evaluated the effects of dexamethasone (Dex) alone or in combination with interleukin (IL)-10 or transforming growth factor-β1 (TGF-β1) on human T cell proliferation. Both IL-10 and TGF-β1 significantly decreased the Dex concentration needed to inhibit T cell proliferation by 50% (IC50). Dex in combination with IL-10 completely inhibited T cell proliferation, even when IL-10 alone was ineffective, as in the case of phytohemagglutinin-induced T cell proliferation. The evaluation of the results according to the isobole method displayed a potent synergistic activity between Dex and IL-10, whereas the combination of Dex with TGF-β1 was additive. IL-10, but not TGF-β1, enhanced the inhibitory effect of Dex on IL-2 production. IL-2 and IL-4 only partly antagonized the antiproliferative effect of the combinations. IL-4 was as effective as IL-2 in antagonizing the combination of Dex with TGF-β1, but significantly less effective against the combination of Dex with IL-10. IL-10 and TGF-β1 are thus able to potentiate the Dex inhibitory effect on T cell proliferation and could be regarded as potential agents for future immunosuppressive protocols.

GC inhibit cytokine production and T cell activation, proliferation and survival (Cups and Fauci, 1982; Barnes and Adcock, 1993; Schwartzmann and Cidlowski, 1994; Brunetti et al., 1995). Because of their effects on the immune response, GC are used in the treatment of allograft rejections, and autoimmune, allergic and inflammatory diseases. Adverse systemic effects are the main disadvantage of long-term GC therapy. They can be dramatic when high doses are required, as in patients who fail to demonstrate a satisfactory response to GC. The adverse effects could be reduced by associating lower doses of GC with other drugs. We have hypothesized that the immunosuppressive properties of two cytokines, namely TGF-β1 and IL-10, could be exploited for this purpose. TGF-β1 inhibits T cell proliferation, deactivates APC (Kehrl et al., 1986; Wahl et al., 1988; Bogdan et al., 1992) and has preventive and curative effects on some experimentally induced autoimmune diseases (Racke et al., 1991; Brandes et al., 1991; Santambrogio et al., 1993). IL-10 inhibits cytokine production and proliferation of T cells by acting on APC and T cells (Moore et al., 1993), and induces T cell anergy (Groux et al., 1996). Administration of IL-10 prevents the induction or decreases the severity of some experimentally induced autoimmune diseases (Rott et al., 1994; Mignon-Godefroy et al., 1995; Walmsley et al., 1996). Furthermore, several lines of evidence suggest that IL-10 plays a role in the maintenance of graft survival (Bromberg, 1995). We have evaluated the effects of Dex alone or in combination with TGF-β1 or IL-10 on human T cell proliferation. Our data indicate that these cytokines potentiate the inhibitory effect of Dex.

Materials and Methods

Cell preparation. PBMC were isolated by Ficoll-Hypaque (Pharmacia Biotech, Brussels, Belgium) density gradient centrifugation from heparinized blood obtained from healthy donors. Purified T lymphocytes were obtained by incubating nonadherent cells with the mAbs anti-CD16 and anti-HLA-DR (Becton Dickinson, Mountain View, CA) and low-tox rabbit complement (Cedarlane Laboratories, Hornby, Ontario, Canada). Ficoll separation was performed to remove dead cells. Cells were more than 90% CD3+ by cytofluorometry. Monocytes were obtained from PBMC by discontinuous Percoll (46%, Pharmacia) density gradient centrifugation and were >85% pure by cytofluorometry. In all experiments, the lymphocyte: monocyte ratio was 10:1, and the culture medium was Roswell Park Memorial Institute 1640 (Gibco Laboratories, Grand Island, NY), supplemented with 2 mM l-glutamine, 100 U/ml of penicillin, 100 µg/ml of streptomycin and 10% (v/v) heat-inactivated fetal calf serum (Gibco).

Proliferation assays. Purified T cells (10⁶/ml) were cultured with mitomycin C-treated (Sigma Chemical Co., St. Louis, MO) au-

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ABBREVIATIONS: GC, glucocorticoids; TGF-β1, transforming growth factor-β1; IL, interleukin; APC, antigen-presenting cells; Dex, dexamethasone; PBMC, peripheral blood mononuclear cells; mAb, monoclonal antibody; PHA, phytohemagglutinin; r, recombinant.
tologous monocytes in the presence of OKT3 mAb (10 ng/ml) (Ortho Pharmaceutical Corp., Raritan, NJ) or PHA (10 μg/ml) (Difco Laboratories, Detroit, MI) in 96-well flat-bottom plates (Falcon; Becton Dickinson) in 200 μl final volume. Dex (Sigma), human rTGF-β1 (Sigma), rIL-10, rIL-2 and rIL-4 (Peprotech Inc., Rocky Hill, NJ) were added at the beginning of the culture at the indicated concentrations. Proliferation was measured at the indicated times by [3H]thymidine (1 μCi/well) (Amersham, Little Chalfont, UK) incorporation.

Evaluation of drug interaction. Dose-response curves were generated for each agent. The extent of the effect of the combination treatment was analyzed by the isobole method (Berenbaum, 1981) for a combination of drugs A and B by the equation:

\[ Ac/Ae + Be/Bc = D \]

where Ac and Be correspond to the concentrations of A and B when used in combination, and Ae and Be to those concentrations able to produce an effect of the same magnitude if used alone. If D (Combination Index) < 1, the effect of the combination is synergistic, whereas if D = 1 or >1, the effect is additive or antagonistic, respectively. The drug Potentiation Index is calculated as the concentration of the drug which, if used alone, would produce the same effect as if it is used in combination, divided by the concentration of drug used in the combination (Ac/Ae or Be/Bc). Student’s t test for paired data was performed to calculate the statistical significance (P) of the Combination Indices (D) compared to the additive Combination Index: D = 1.

Assays for IL-2 and IL-4 production. Purified T cells (10⁶/ml) were cultured with or without OKT3 mAb (10 ng/ml) in the presence of mitomycin C-treated autologous monocytes for 24 hr, with or without the indicated concentrations of Dex, TGF-β1 and IL-10. IL-2 and IL-4 were measured in the supernatants with IL-2 (sensitivity <6 pg/ml) and IL-4 (sensitivity <2 pg/ml)-specific enzyme-linked immunosorbent assay kits (Amersham).

Northern analysis. Purified T cells (10⁶/ml) were cultured with or without OKT3 mAb (10 ng/ml) in the presence of mitomycin C-treated autologous monocytes for 24 hr, with or without the indicated concentrations of Dex, TGF-β1 and IL-10. Total RNA was extracted by the guanidine-isothiocyanate-CsCl gradient method, size-fractioned on denaturing 1% agarose gel and transferred to nylon membrane filters by capillary blotting. The blots were hybridized overnight with 32P-labeled cDNAs for human IL-2 (Sst I, Hind III, 550 bp) from Dr. C. Mills and 18SrRNA (Hind III, 3000 bp) from Dr. Nikki Holbrook, National Institute of Aging, Baltimore, MD, and 18S rRNA (Hind III, 3000 bp) from Dr. C. Milcarek, University of Pittsburgh, Pittsburgh, PA. After hybridization, filters were washed and autoradiographed at −80°C using Kodak XAR film (Eastman Kodak, Rochester, NY).

Statistical analysis. Results are means ± S.D. Student’s t test for paired data was performed for statistical analysis.

Results

Effect of Dex in combination with TGF-β1 or IL-10 on T cell proliferation. Dex (1–1000 nM), TGF-β1 and IL-10 (0.0005–0.5 nM) inhibited anti-CD3-induced T cell proliferation in a dose-dependent manner (fig. 1, A and B). The maximal inhibitory activities (without cytotoxic effects, as assessed by trypan blue exclusion test) were obtained with 1.000 nM Dex, 0.5 nM TGF-β1 and 0.5 nM IL-10, and were 46 ± 4, 41 ± 7 and 25 ± 4% of control, respectively (control proliferation = 85,718 ± 11,367 cpm) (fig. 1, A and B). When Dex was combined with TGF-β1 or IL-10, a dose-dependent enhancement of the Dex inhibitory effect was observed (fig. 1, A and B). TGF-β1 and IL-10 significantly decreased the Dex concentration needed to inhibit T cell proliferation by 50% (IC50) (table 1). Dex in combination with IL-10 completely inhibited T cell proliferation, whereas in combination with TGF-β1 it inhibited it by up to 31 ± 5% (fig. 1, A and B). We then evaluated the results according to the isobole method (Berenbaum, 1981). The combination of Dex with TGF-β1 acted in an additive manner (Combination Index = 1) (table 2). A significant synergistic activity (Combination Index <1) was observed only in the presence of 100 nM Dex in combination with 0.05 nM TGF-β1 (table 2). A potent synergistic activity between Dex and IL-10 was observed at 1 nM Dex in combination with 0.05 nM IL-10, and at both 10 and 100 nM Dex in combination with 0.005 and 0.05 nM IL-10 (table 2). The Dex Potentiation Index ranged from 4 to 532 for the combination of Dex with TGF-β1, and from 8 to >1000 for the combination of Dex with IL-10 (table 2).

Dex (1–1000 nM), TGF-β1 and IL-10 (0.0005–0.5 nM), alone or in combination, were tested on PHA-induced (10 μg/ml) T cell proliferation. The combinations of Dex with TGF-β1 or IL-10 were more inhibitory than Dex alone (fig. 2, A and B), and the Dex IC50 was significantly decreased by TGF-β1 and IL-10 (data not shown). It has been reported that IL-10 alone does not inhibit T cell proliferation induced by high PHA concentrations (Taga and Tosato, 1992). Although IL-10 alone did not inhibit the PHA-induced T cell proliferation, when combined with Dex it completely inhibited it (fig. 2B). Dex, TGF-β1 and IL-10, alone or in combination, did not delay the peak of anti-CD3- or PHA-induced T cell proliferation, nor did they decrease T cell viability, as assessed by trypan blue exclusion at 24, 48 and 72 hr (data not shown).

Fig. 1. Effect of Dex in combination with TGF-β1 or IL-10 on anti-CD3-induced T cell proliferation. A: T cells were cultured with anti-CD3 mAb (10 ng/ml) in the presence of autologous mitomycin C-treated monocytes in medium alone or supplemented with Dex, TGF-β1 or Dex plus TGF-β1 at the indicated concentrations. B: Cells stimulated as in (A) were cultured in medium alone or supplemented with Dex, IL-10 or Dex plus IL-10 at the indicated concentrations. After 72 hr, proliferation was measured by [3H]thymidine incorporation. Results from 4 experiments performed in triplicate are expressed as the mean percentage of control (control proliferation = 85,718 ± 11,367 cpm). S.D.s were less than 12% and are omitted.
TABLE 1

<table>
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<th>Cytokine Concentration (nM)</th>
<th>IC₅₀</th>
<th>IC₅₀</th>
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<tr>
<td>0</td>
<td>393 ± 57</td>
<td>393 ± 57</td>
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<tr>
<td>0.0005</td>
<td>361 ± 56*</td>
<td>360 ± 61*</td>
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<tr>
<td>0.0500</td>
<td>77 ± 2**</td>
<td>30 ± 2**</td>
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<tr>
<td>0.0500</td>
<td>12 ± 1***</td>
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<tr>
<td>0.5000</td>
<td>5 ± 2</td>
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</table>

* Results from one of four experiments are expressed as means ± S.D. of triplicate determinations. IC₅₀ = the concentration resulting in a 50% inhibition of T cell proliferation.
* P = N.S.; ** P < .01.

TABLE 2

<table>
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<tr>
<th>Cytokine</th>
<th>Potentiation Indices for the effect of Dex in combination with TGF-β1 or IL-10 on anti-CD3-induced T cell proliferation</th>
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<td>TGF-β1</td>
</tr>
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<td>nM</td>
<td></td>
</tr>
<tr>
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<td>0.005</td>
</tr>
<tr>
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<td>0.005</td>
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<tr>
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</table>

* Combination Index (D) = Ac/Dc + Bc/Be, where Ac and Be correspond to the concentrations of Dex (Ac) and TGF-β1 or IL-10 (Be) when used in combination, and Ac and Bc to those concentrations able to produce an effect of the same magnitude if used alone. Dex Potentiation Index = the concentration of Dex which, if used alone, would produce the same effect as if it were used in combination with TGF-β1 or IL-10, divided by the concentration of Dex used in combination. Results are the means of four experiments. Statistical significance (P) was calculated comparing D values to the additive Combination Index (D = 1) by a one-sided paired t test.
* P = N.S.; ** P < .05; *** P < .001.

**Effect of Dex in combination with TGF-β1 or IL-10 on IL-2 production and mRNA expression.** We tested the effect of Dex (1–100 nM), alone or in combination with TGF-β1 and IL-10 (0.005–0.5 nM), on anti-CD3-induced IL-2 production. When the Dex concentration was increased from 1 to 100 nM, IL-2 production decreased from 90 ± 6 to 53 ± 3% of control (control IL-2 production = 884 ± 61 pg/ml) (fig. 3A). IL-10, but not TGF-β1, increased the inhibitory effect of Dex in a dose-dependent manner; Dex (100 nM) in combination with IL-10 (0.5 nM) reduced IL-2 production almost to the basal level (fig. 3A). IL-10 alone at concentrations from 0.005 to 0.5 nM decreased IL-2 production from 80 ± 5 to 27 ± 3% of control, whereas TGF-β1 alone was ineffective. The anti-CD3-induced IL-2 mRNA expression was inhibited partially by Dex or IL-10 alone, whereas their combination reduced it almost to the basal level. TGF-β1 did not inhibit IL-2 mRNA expression, nor did it modify the inhibitory effect of Dex (fig. 3B).

**Effect of IL-2 and IL-4 on the antiproliferative activity of the combinations of Dex with TGF-β1 or IL-10.** We investigated whether exogenous IL-2 or IL-4 could antagonize the inhibitory effect of Dex, TGF-β1, and IL-10 alone or in combination. The effects of IL-2 (0.1–10 ng/ml) and IL-4 (0.1–100 ng/ml) were dose-dependent, and were maximal at 2 and 20 ng/ml, respectively (data not shown). Addition of IL-2 (2 ng/ml) to cultures containing 100 nM Dex, 0.5 nM TGF-β1, or Dex plus TGF-β1 significantly increased (P < .01) T cell proliferation from 51 ± 14 to 92 ± 10, from 42 ± 13 to 75 ± 13 and from 34 ± 13 to 55 ± 13% of control, respectively (control proliferation = 94,563 ± 13,840 cpm) (fig. 4). Addition of IL-2 to cultures containing 0.5 nM IL-10 or IL-10 significantly increased (P < .01) T cell proliferation from 21 ± 10 to 96 ± 16, and from 1 ± 1 to 66 ± 15% of control, respectively (fig. 4). IL-4 (20 ng/ml) was as effective as IL-2 in counteracting the effect of Dex plus TGF-β1, but it was significantly less effective than IL-2 (P < .01) against Dex plus IL-10 (fig. 4).

**Discussion**

Our study shows that both IL-10 and TGF-β1 enhance the inhibitory effect of Dex on T cell proliferation in a dose-dependent manner. The combination of Dex with IL-10 is synergistic and completely inhibits T cell proliferation even when, in the presence of a high PHA concentration, IL-10 alone is ineffective. One of the mechanisms underlying the effect of Dex plus IL-10 could be the inhibitory effect on IL-2 production and mRNA expression. It is known that Dex decreases IL-2 production (Cups and Fauci, 1982) by inhibiting the binding of the transcription factors AP-1, NF-AT and NF-kB to the IL-2 promoter (Vacca et al., 1992; Paliogianni et al., 1993b; Auphan et al., 1995). It also decreases IL-2 mRNA stability (Boumpas et al., 1991). The inhibitory activity of IL-10 on IL-2 production seems mainly the consequence of the inhibition of APC accessory activity (Moore et al., 1993; Ding and Shevach, 1992; Caux et al., 1994), although a direct inhibitory effect on IL-2 production by T cells, in the absence of APC, has been reported (Taga et al., 1993; de Waal Malefyt et al., 1993). In addition, IL-10 inhibits NF-kB activity in CD3-stimulated T cells (Romano et al., 1996). All these inhibitory pathways could converge in reducing IL-2 production. Exogenous IL-2, however, does not completely reconstitute the T cell response inhibited by Dex plus IL-10, suggesting that the effect of this combination cannot be attributed solely to the inhibition of IL-2 production. An inhibition of signal transduction pathways downstream of IL-2 receptor binding (Paliogianni et al., 1993a) could also be taken into consideration. In agreement with previous reports (Holter et al., 1992; Conlon et al., 1995), we have found that IL-4 is undetectable or present in trace amounts in the supernatants of OKT3-stimulated freshly isolated T cells (data not shown). IL-4 added to cultures containing Dex or IL-10 alone substantially reconstitutes T cell proliferation, but it is poorly effective in antagonizing the effect of Dex in combination with IL-10. As IL-4 plays an important role in the pathogenesis of atopic diseases (Daser et al., 1995), the combination of Dex with IL-10 could be tested in animal models of these diseases.

The combination of Dex with TGF-β1 acts in an additive manner. TGF-β1 does not affect IL-2 production (Wahl et al., 1988; Ahuja et al., 1993). Our data extend these observations by demonstrating that it does not increase the inhibitory effect of Dex on IL-2 production. TGF-β1 arrests cells in the G1 phase of the cell cycle by inhibiting the activity of cyclin-dependent kinases (Ahuja et al., 1993; Saltis, 1996). It may perhaps cooperate with Dex by down-regulating the activity.
of these components of the cell cycle machinery. Although TGF-β1 fails to inhibit IL-2 production, addition of IL-2 partly reverts the effect of TGF-β1 on T cell proliferation. This could depend on the IL-2 inactivating effect on cyclin-dependent kinase inhibitors (Nourse et al., 1994). IL-4, which is less effective than IL-2 in antagonizing the combination of Dex with IL-10, is as effective as IL-2 in antagonizing the combination of Dex with TGF-β1. This further supports the view that different mechanisms underlie the effects of the two combinations.

T cells play a pivotal role in transplant rejection, hypersensitivity reactions and autoimmune diseases. All these conditions are currently treated with GC. We have shown that the combination of Dex with IL-10 synergistically blocks T cell proliferation. This result, together with the observation that significant IL-10 concentrations have been attained in human serum without side effects (Chernoff et al., 1995; Huhn et al., 1996; Pajkrt et al., 1997), suggests that IL-10 may be useful for the potentiation of GC therapy. TGF-β1 potentiates Dex in an additive manner; thus, it may be of therapeutic significance. Our work, together with reports showing the efficacy of IL-10 or TGF-β1 in animal models of...

Fig. 2. Effect of Dex in combination with TGF-β1 or IL-10 on PHA-induced T cell proliferation. A: T cells were cultured with PHA (10 μg/ml) in the presence of autologous mitomycin C-treated monocytes in medium alone or supplemented with Dex, TGF-β1 or Dex plus TGF-β1 at the indicated concentrations. B: Cells stimulated as in (A) were cultured in medium alone or supplemented with Dex, IL-10 or Dex plus IL-10 at the indicated concentrations. After 72 hr, proliferation was measured by [3H]thymidine incorporation. Results from 4 experiments performed in triplicate are expressed as the mean percentage of control (control proliferation = 121,908 ± 8,204 cpm). S.D.s were less than 13% and are omitted.

Fig. 3. Effect of Dex in combination with TGF-β1 or IL-10 on IL-2 production and mRNA expression. A: T cells were cultured with anti-CD3 mAb (10 ng/ml) in the presence of autologous mitomycin C-treated monocytes for 24 hr, in medium alone or supplemented with Dex, or Dex plus TGF-β1 or IL-10 at the indicated concentrations. IL-2 levels were assayed in the supernatants by enzyme-linked immunosorbent assay. Results from 1 of 3 experiments performed in triplicate are expressed as the mean ± S.D. percentage of control (control IL-2 production = 884 ± 61 pg/ml). B: T cells prepared as in (A) were cultured for 24 hr in either medium alone; anti-CD3 mAb (10 ng/ml); anti-CD3 mAb plus Dex (100 nM), TGF-β1 or IL-10 (0.5 nM); or anti-CD3 mAb plus Dex (100 nM) in combination with TGF-β1 or IL-10 (0.5 nM). Total mRNA (10 μg/lane) was examined by Northern analysis. 18S ribosomal RNA expression is shown as RNA loading control. Results are from one of three experiments.

Fig. 4. Effect of IL-2 and IL-4 on the antiproliferative activity of Dex in combination with TGF-β1 or IL-10. T cells were cultured with anti-CD3 mAb (10 ng/ml) in the presence of autologous mitomycin C-treated monocytes in medium alone or supplemented with Dex (100 nM), TGF-β1 or IL-10 (0.5 nM) or Dex (100 nM) plus TGF-β1 or IL-10 (0.5 nM). These cultures were performed with or without IL-2 (2 ng/ml) or IL-4 (20 ng/ml). After 72 hr, proliferation was measured by [3H]thymidine incorporation. Results from 5 experiments performed in triplicate are expressed as the mean ± S.D. percentage of control (control proliferation = 94,563 ± 13,840 cpm).
alloreactivity (Delvaux et al., 1994; Quin et al., 1995) and autoimmune diseases, suggests that further studies, designed to establish cytokine-based therapeutic protocols, could be usefully undertaken.

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References


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