Modulation of Graves' Orbital Fibroblast Proliferation by Cytokines and Glucocorticoid Receptor Agonists

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Purpose. Paracrine/autocrine interactions between orbital fibroblasts (OF) and infiltrating lymphocytes/macrophages are thought to play a central role in the evolution of Graves' ophthalmopathy (GO). Compounds capable of stimulating the proliferation and synthetic capacities of OF may be of particular importance to these processes, because fibroblasts are known to both produce and respond to certain paracrine factors.

Methods. The effects of interleukin-1 alpha, interleukin-2, interleukin-4, interleukin-6, insulin-like growth factor I, transforming growth factor beta, and platelet-derived growth factor on OF monolayers derived from orbital fatty connective tissue and extraocular muscle ends from patients with severe GO undergoing orbital decompression (n = 3), and from connective tissue of normal persons (n = 3) were investigated. Stimulation of proliferation in growth-arrested OF was determined using immunocytochemical staining for the cell-proliferation-related nuclear antigen recognized by a monoclonal anti-Ki 67 antibody. In addition, the effects of OF coinubation with one of the aforementioned compounds and hydrocortisone (10⁻⁷ M), the selective glucocorticoid receptor agonist RU 28362 (10⁻⁷ M), or the glucocorticoid receptor antagonist RU 38486 (10⁻⁷ M) were assessed.

Results. Under baseline conditions (0.1% fetal bovine serum), the proportion of proliferating cells was significantly higher in GO-OF compared with normal OF (p < 0.001). Significant stimulation of GO-OF proliferation was observed with interleukin-1 alpha (10 U/ml), interleukin-4 (1 ng/ml), insulin-like growth factor I (10 ng/ml), transforming growth factor beta (10 ng/ml), platelet-derived growth factor (1 ng/ml), and 1% or 15% fetal bovine serum (all P < 0.01), but not with interleukin-2 (10 U/ml) and interleukin-6 (100 U/ml). Compared with GO-OF, proliferation of normal OF was stimulated by fetal bovine serum to a similar degree, by interleukin-4, insulin-like growth factor I, transforming growth factor beta, and platelet-derived growth factor to a significantly lesser degree (all P < 0.01), and was unaffected by interleukin-1 alpha, interleukin-2, and interleukin-6. Compared with normal OF, either glucocorticoid receptor agonists, but not testosterone or progesterone, specifically inhibited the cytokine-stimulated proliferation of GO-OF to a significantly greater degree (P < 0.01).

Conclusions. The enhanced proliferative capacity of GO-OF at baseline and in response to certain cytokines could play a role in the evolution of the clinical manifestations in GO. Inhibition of cytokine-activated cellular functions may be one mechanism by which glucocorticosteroids exert clinically useful effects in GO. Invest Ophthalmol Vis Sci. 1994;35:120-127

Infiltration of the retroocular space by inflammatory cells and the accumulation of hydrophilic glycosaminoglycans are histologic hallmarks of Graves' ophthalmopathy (GO).1-4 These features lead to the clinical manifestations of proptosis, diplopia, periorbital swelling, and inflammation.5 Various cytokines, produced by infiltrating monocyes/macrophages, mast cells, and lymphocytes, as well as resident connective tissue cells, are considered to act as local modulators of cel-
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Cytokine effects of potential relevance to the orbital disease process in GO include, among others, the induction of major histocompatibility complex class II molecules, heat shock proteins, and adhesion molecules in retroorbital fibroblasts. These cells are likely both target and effector cells in the ongoing immune process in GO. In addition, the production of glycosaminoglycans (predominantly hyaluronate) by retroorbital fibroblasts is stimulated by interferon gamma, interleukin-1 alpha (IL-1α) and transforming growth factor-beta (TGFβ). Glucocorticoid treatment of retroocular fibroblasts in vitro has been shown to be effective in modulating immunologic and metabolic properties of these cells, including cytokine-induced expression of HLA-DR molecules and synthesis of glycosaminoglycans. Although of potential relevance to the increased volume of the orbital contents in GO and to the impairment of extraocular muscle function, the mechanisms affecting the proliferation of orbital fibroblasts have not been studied in detail. The proliferative capacity of these fibroblasts may be under the influence of locally produced cytokines and growth factors, and modulation of cell proliferation may be one mechanism by which glucocorticoids exert their clinical benefits in GO. The current study was designed to determine if certain cytokines and growth factors are capable of stimulating retroocular fibroblast proliferation, and if these effects might be modulated by glucocorticoid treatment. Our results demonstrate that several cytokines are potent stimulators of orbital fibroblast proliferation and that inhibition of fibroblast proliferation by glucocorticoid agonists involves a glucocorticoid receptor-mediated mechanism of action.

**MATERIALS AND METHODS**

**Reagents**

Recombinant cytokines were purchased from Genzyme Corporation (Boston, MA) and Boehringer Mannheim (Indianapolis, IN). Neutralizing polyclonal anti-cytokine antibodies were obtained from Genzyme. Hydrocortisone, testosterone, and progesterone were from Sigma Chemical Company (St. Louis, MO). RU 28362 and RU 38486 were donated by Roussel UCLAF (Romainville, France). Monoclonal anti-Ki 67 antibody was purchased from Dakopatts (Glostrup, Denmark). Biotinylated anti-mouse immunoglobulin G antibody and the Vectastain ABC Elite detection system were purchased from Vector Laboratories (Burlingame, CA).

**Cell Culture**

Orbital connective tissue was obtained as surgical waste from patients during transantral orbital decompression for severe GO (n = 4). All patients had had hyperthyroid Graves’ disease and had been rendered euthyroid before transantral orbital decompression. Patients had received glucocorticoids (60 to 80 mg per day, followed by tapering of doses) in the distant past, but not within 4 months before transantral orbital decompression. Normal retroocular connective tissue was obtained from three persons undergoing orbital surgery for non-GO, noninflammatory conditions. Endomyinal connective tissue was obtained from extraocular muscles during surgery to correct diplopia in GO patients (n = 3) and during strabismus surgery in normal persons (n = 3). Orbital fibroblast (OF) monolayers were established and propagated in medium 199 containing fetal bovine serum (FBS; Hyclone Laboratories, Logan, UT) (10%, pH 7.25) and antibiotics as described previously. All cell strains were used between the third and fifth cell passages. For proliferation studies, equal cell numbers of OF were plated directly onto 8 × 8 mm multichamber slides (Nunc, Naperville, IL), resulting in cell monolayers of equal densities (approximately 80% confluency) after adherence of the cells. Monolayers were switched to medium 199 containing 0.1% FBS for 24 hours and growth-arrested in medium 199 without serum during the final 24 hours before incubation for 72 hours with the compounds indicated. Agents were diluted in medium 199 containing 0.1% FBS to these concentrations: IL-1α (10 U/ml), interleukin-2 (10 U/ml), interleukin-4 (IL-4; 1 ng/ml), interleukin-6 (100 U/ml), insulin-like growth factor 1 (10 ng/ml), TGFβ (10 ng/ml) and platelet-derived growth factor (PDGF; 1 ng/ml). Previous experiments, designed to determine the dose dependency of retroorbital fibroblast responses to cytokines, had revealed maximal stimulation of cell proliferation at these concentrations. Further, as demonstrated in our earlier studies, these cytokine concentrations have been found to maximally stimulate various other important effects in retroocular fibroblasts, including the synthesis of glycosaminoglycans and the expression of various immunomodulatory molecules. In some experiments, monolayers were coincubated with neutralizing polyclonal antibodies directed against IL-1α, PDGF, IL-4, and TGFβ, with the selective glucocorticoid receptor agonist RU 28362 (10 M), testosterone (10 M), progesterone (10 M), or the glucocorticoid receptor antagonist RU 38486 (10 M). Neutralizing anticytokine antibodies and steroid hormones were used at predetermined maximally inhibitory and stimulatory concentrations, respectively.

**Immunocytochemistry**

Cell proliferation was determined immunocytochemically using an anti-Ki 67 monoclonal antibody. This antibody reacts with a human nuclear antigen that is
expressed by all proliferating cells during late G1, S, M, and G2 phases of the cell cycle. Its suitability for cell proliferation studies, using both immunocytochemical and immunohistochemical detection protocols, has been validated and reviewed extensively. Immunoreactivity was detected using a highly sensitive three-stage immunoperoxidase protocol, as described previously. In brief, viable monolayers (trypan blue exclusion in >95% of cells) were fixed in 100% cold acetone for 10 minutes, and endogenous peroxidase was blocked with cold methanol for 30 minutes. Non-specific binding was blocked by pretreatment of monolayers with normal horse serum, diluted 1:20 in phosphate-buffered saline, for 30 minutes. Anti-Ki-67 mouse monoclonal antibody was applied at 1:50 dilution in phosphate-buffered saline containing 3% normal horse serum and Tween-20 (0.05%) for 1 hour at room temperature. Washed monolayers were incubated with biotinylated anti-mouse immunoglobulin at 1:250 dilution in phosphate-buffered saline, pH 7.4, containing 1% normal horse serum) for 30 minutes at room temperature. Slides were rinsed extensively and incubated with avidin and biotinylated peroxidase for 30 minutes at room temperature, and rinsed again with phosphate-buffered saline containing Tween-20. Immunoreactivity was detected using a standard peroxidase substrate system containing diaminobenzidine as the chromogen. A brown nuclear immunoprecipitate was indicative of Ki-67-positive staining. Parallel monolayers with the primary antibody replaced by nonimmune immunoglobulin G, by irrelevant monoclonal antibodies of the same isotype, and by omitting the primary and secondary antibody in turn, were examined to ensure specificity of staining and to exclude cross-reactivities between the antibodies and conjugates employed. The percentage of proliferating cells was determined using optical microscopy and differential cell counting. Nuclear staining was graded as either positive or negative for the Ki-67 antigen. Triplicate experiments were performed for each treatment condition. At least 250 individual cells in four randomly selected visual fields were evaluated. The examiner was blinded as to the treatment employed.

To compare our results (using Ki-67 immunostaining) with other methods for the determination of cell proliferation, parallel experiments were performed using a standard tritiated thymidine assay, an immunocytochemical assay using a monoclonal antibody directed against the proliferating cell nuclear antigen, and an automated colorimetric enzyme-linked immunosorbent assay (Promega, Madison, WI). With any of these techniques, no significant differences were found (data not shown). Investigations followed the tenets of the Declaration of Helsinki. Informed consent was obtained from the subjects after the nature and possible consequences of the study were explained to them. The research protocol was approved by the institutional human experimentation committee.

Statistical Analysis

Unless stated otherwise, all experiments were performed in triplicate. Results are given as the mean (± SD) of percent proliferating cells. Student’s t test for the analysis of paired and unpaired data was used to assess the level of statistical significance. P values of 0.05 or less were considered statistically significant.

RESULTS

Under baseline conditions (medium 199 supplemented with 0.1% FBS), the proportions of cells displaying nuclear Ki-67 immunoreactivity were significantly greater in GO-OF (n = 3) compared with normal OF (n = 3) (P < 0.001; Table 1). No differences were observed between fibroblasts derived from the orbital connective tissue and those derived from extracocular muscle perimysium of patients with GO. A 72-hour incubation of growth-arrested monolayers in medium 199 supplemented with either 1% or 15% FBS markedly stimulated the proportion of proliferating cells in both GO-OF and normal OF (all P < 0.0001). Under these culture conditions, no significant differences were detected between GO-OF and normal OF.

The effects of cytokines on OF proliferation are shown in Figure 1. Treatment of monolayers with IL-4 (1 ng/ml), TGFβ (10 ng/ml), insulin-like growth factor 1 (1 ng/ml), and PDGF (1 ng/ml) significantly stimulated cell proliferation both in GO-OF and normal OF (all P < 0.0001). No significant differences were observed between fibroblasts derived from the orbital connective tissue and those derived from extracocular muscle perimysium of patients with GO. However, after incubation with IL-4 (P < 0.05), TGFβ (P < 0.01), and PDGF (P < 0.01), significantly more GO-OF than normal OF revealed immunoreactivity for the nuclear Ki 67 antigen. Treatment with interleukin-2 (10 U/ml) or interleukin-6 (100 U/ml) failed to stimulate proliferation in growth-arrested GO-OF or normal OF (data not shown).

Treatment of monolayers with IL-1α resulted in marked stimulation of cell proliferation in GO-OF (P < 0.0001), whereas only minimal stimulation occurred in normal OF (Figure 2 and Table 1). To further study if this difference in cell proliferation represents a direct response to IL-1α or involves another stimulating factor, a series of experiments was performed using neutralizing antibodies directed against several proliferation-promoting cytokines. As demonstrated in Figure 3, the stimulatory effect of IL-1α on GO-OF proliferation was blocked by an antibody that neutralizes IL-1α activity (P < 0.0001), and markedly reduced by...
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costeroids involve a modulatory action on cytokine-
stimulated OF proliferation, a series of coincubation
TGFβ + RU 28362 + RU
TGFβ + HC
TGFβ alone
IL-1α + testosterone
IL-1α + progesterone
IL-1α + RU 28362 + RU 38486
IL-4 alone
IL-4 + HC
IL-4 + RU 28362
IL-4 + progesterone
IL-4 + RU 38486 + RU 38486
IGF-I alone
IGF-I + HC
IGF-I + RU 28362
IGF-I + RU 38486 + RU 38486
TGFβ alone
TGFβ + HC
TGFβ + RU 38486
TGFβ + testosterone
TGFβ + progesterone
TGFβ + RU 3846 + RU 3846
PDGF alone
PDGF + HC
PDGF + RU 28362
PDGF + testosterone
PDGF + progesterone
PDGF + RU 3846 + RU 3846

Growth-arrested monolayers were incubated for 72 hours with medium 199 containing 0.1% FBS and the agonists indicated. Concentrations were as follows: hydrocortisone (HC); testosterone; progesterone; RU 28362; RU 38468 (all 10⁻⁷ M); IL-1α (10 U/ml); IL-4 (1 ng/ml); IGF-I (10 ng/ml); TGFβ (10 ng/ml); PDGF (1 ng/ml). The proportions of proliferating cells were determined immunochemically using an anti-Ki 67 monoclonal antibody. Data represent the mean of % proliferating cells ± SD, derived from triplicate experiments.

TABLE 1. Modulation of Cytokine-stimulated Retroocular Fibroblast Proliferation by Steroid Compounds

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GO-OF</th>
<th>Normal OF</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS (0.05%)</td>
<td>3 ± 1</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>FBS (0.1%)</td>
<td>11 ± 4</td>
<td>3 ± 2</td>
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<tr>
<td>FBS (1%)</td>
<td>48 ± 14</td>
<td>34 ± 11</td>
</tr>
<tr>
<td>FBS (15%)</td>
<td>89 ± 17</td>
<td>76 ± 16</td>
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<tr>
<td>Hydrocortisone</td>
<td>7 ± 3</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>RU 28362</td>
<td>7 ± 2</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Testosterone</td>
<td>10 ± 2</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>Progesterone</td>
<td>18 ± 6</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>RU 38486</td>
<td>0 ± 2</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>IL-1α alone</td>
<td>50 ± 11</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>IL-1α + HC</td>
<td>22 ± 6</td>
<td>7 ± 3</td>
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<tr>
<td>IL-1α + RU 28362</td>
<td>16 ± 7</td>
<td>6 ± 3</td>
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<tr>
<td>IL-1α + TGFβ</td>
<td>54 ± 12</td>
<td>14 ± 5</td>
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<tr>
<td>IL-1α + progesterone</td>
<td>61 ± 14</td>
<td>12 ± 3</td>
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<tr>
<td>IL-1α + RU 3846 + RU 3846</td>
<td>47 ± 12</td>
<td>10 ± 4</td>
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<td>IL-4 alone</td>
<td>43 ± 12</td>
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<td>IL-4 + HC</td>
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<td>IL-4 + RU 3846</td>
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<td>17 ± 4</td>
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<tr>
<td>IL-4 + testosterone</td>
<td>46 ± 8</td>
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<td>IL-4 + progesterone</td>
<td>49 ± 7</td>
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<td>IL-4 + RU 3846 + RU 3846</td>
<td>44 ± 9</td>
<td>31 ± 7</td>
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<td>IGF-I alone</td>
<td>38 ± 7</td>
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<td>IGF-I + HC</td>
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<td>IGF-I + RU 28362</td>
<td>30 ± 7</td>
<td>28 ± 6</td>
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<td>IGF-I + progesterone</td>
<td>51 ± 11</td>
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<td>IGF-I + RU 3846 + RU 3846</td>
<td>40 ± 9</td>
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<td>TGFβ alone</td>
<td>58 ± 12</td>
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<td>TGFβ + testosterone</td>
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<td>TGFβ + progesterone</td>
<td>71 ± 13</td>
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<td>TGFβ + RU 3846 + RU 3846</td>
<td>55 ± 12</td>
<td>31 ± 8</td>
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<tr>
<td>PDGF alone</td>
<td>62 ± 13</td>
<td>45 ± 9</td>
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<td>PDGF + HC</td>
<td>28 ± 8</td>
<td>24 ± 6</td>
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<td>PDGF + RU 3846</td>
<td>25 ± 6</td>
<td>22 ± 6</td>
</tr>
<tr>
<td>PDGF + testosterone</td>
<td>58 ± 11</td>
<td>44 ± 11</td>
</tr>
<tr>
<td>PDGF + progesterone</td>
<td>74 ± 12</td>
<td>48 ± 11</td>
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<tr>
<td>PDGF + RU 3846 + RU 3846</td>
<td>56 ± 9</td>
<td>44 ± 10</td>
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DISCUSSION

Accumulation of hydrophilic macromolecules and the attendant interstitial edema are characteristic histologic features of the retrobulbar tissues in GO,14 Mononuclear cell infiltration can also be observed to a variable degree, affecting largely the extraocular muscles and their surrounding connective tissue.6,30,31

an antibody that neutralizes PDGF activity (P < 0.001). In contrast, incubation with neutralizing antibodies directed against IL-4 and TGFβ failed to alter the degree of IL-1α-enhanced GO-OF proliferation.

To study if the clinically beneficial effects of corticosteroids involve a modulatory action on cytokine-stimulated OF proliferation, a series of coincubation experiments was performed. To this effect, OF monolayers were treated simultaneously with cytokines and hydrocortisone, RU 28362, testosterone, or progesterone for 72 hours before anti-Ki 67 staining. The results from these experiments are shown in Table 1. Either glucocorticoid receptor antagonist failed to significantly alter the degree of cell proliferation observed under baseline conditions (medium 199 containing 0.1% FBS), but was capable of significantly reducing the stimulatory effect of IL-1α, IL-4, TGFβ, and PDGF on OF proliferation (all P < 0.001). By contrast, hydrocortisone and RU 28362 did not alter OF proliferation in monolayers stimulated by insulin-like growth factor 1. The inhibitory effect of either corticosteroids on cytokine-enhanced cell proliferation was significantly stronger in GO-OF compared with normal OF (P < 0.01 for IL-1α; P < 0.05 for IL-4; P < 0.0001 for TGFβ; P < 0.01 for PDGF). Both testosterone and progesterone failed to significantly reduce the degree of cytokine-stimulated OF proliferation, suggesting that the inhibitory effect of hydrocortisone and RU 28362 specifically involves the glucocorticoid receptor. In contrast, in some instances, enhancement by progesterone of the stimulatory activity of certain cytokines on GO-OF proliferation was noted (Table 1).

To further examine if the inhibitory action of corticosteroids on cytokine-stimulated OF proliferation is mediated by glucocorticoid receptors present in these cells,27 monolayers exposed to medium 199 containing 0.1% FBS, one of several cytokines/growth factors and hydrocortisone or the specific glucocorticoid receptor agonist RU 2836228 were coincubated with the glucocorticoid receptor antagonist RU 3846.29 As demonstrated in Table 1, coadministration of RU 3846 was capable of completely blocking the inhibitory effects of either hydrocortisone or RU 28362 on the proliferation of OF after stimulation with IL-1α, IL-4, TGFβ, or PDGF. In contrast, the stimulatory effect of insulin-like growth factor I on OF proliferation was only minimally inhibited by either one of these compounds. Further, either glucocorticoid agonist (10⁻⁷ M) inhibited cytokine-stimulated cell proliferation in GO-OF to a significantly greater extent than in normal OF (P < 0.0001 for IL-1α; P < 0.01 for IL-4; P < 0.001 for TGFβ; P < 0.01 for PDGF).
Both the interstitial connective tissue surrounding extraocular muscle fibers and fibroblasts within the orbital fatty connective tissue remote from extraocular muscle have been implicated as targets of the immune process in GO. It has been suggested that the evolution of the clinical manifestations in GO results from the interaction of various inflammatory mediators within the orbit that are released by activated mononuclear cells during the ongoing immune process. We have recently demonstrated both the presence of several cytokines in situ in GO tissues and the production of cytokines by tissue explants and in consecutive passages of retroocular fibroblasts derived from patients with severe GO. These studies have suggested that certain cytokines are predominantly released from the infiltrating inflammatory cells. Other cytokines (such as IL-1α) are capable of altering metabolic activities of retroocular fibroblasts in a paracrine and autocrine manner and are also produced by fibroblasts within the orbital connective tissue. Similar results have been reported in studies using fibroblasts from other anatomic sources, indicating that fibroblasts are capable of producing and responding to a number of cytokines, including TNFα, IL-1α, IL-4, and TGFβ.

In this study, we demonstrated that IL-1α, IL-4, insulin-like growth factor I, TGFβ, and PDGF are capable of stimulating the proliferation of fibroblasts derived both from the retroocular connective tissue and from the extraocular muscle perimysium of patients with GO. By contrast, some of the cytokines tested (interleukin-2, interleukin-6) did not affect cell proliferation in these cells, and IL-1α was effective in GO-OF, but not in normal OF. Pretreatment with hydrocortisone and with the specific glucocorticoid receptor agonist RU 38262, but not with testosterone or progesterone, diminished the stimulatory effect of certain cytokines on retroocular fibroblast proliferation. In addition, the effects of hydrocortisone and RU 38262 were inhibited by coadministration of the glucocorticoid receptor antagonist RU 38486. These results indicate that the inhibitory effect of steroids is restricted to glucocorticoid receptor agonists and suggest the involvement of a glucocorticoid receptor-mediated mechanism of action. Further, in addition to their capacity of modulating cytokine gene expression at the transcriptional level, glucocorticosteroids appear to be effective inhibitors of cytokine-mediated cellular responses.

Differences in the cellular proliferative response to both stimulation by cytokines and inhibition by corticosteroids were observed depending on whether cells were derived from patients with GO or from normal person. Compared with normal OF, GO-OF revealed significantly greater responsiveness to both stimulatory and inhibitory stimuli of cell proliferation. In particular, IL-1α induced a marked increase in GO-OF proliferation, an effect that is mediated, at least in part, through PDGF. These results agree with previously reported studies demonstrating involvement of a PDGF-mediated autocrine loop in the stimulation of fibroblast proliferation by IL-1α and TGFβ. In contrast, IL-1α failed to significantly alter cell proliferation in normal OF. Fibroblast derived from the orbital connective tissue or from extraocular muscle endomysium were similar in their proliferative response to stimulating or inhibitory compounds. Thus, although OF were derived from the highly selected group of patients requiring orbital decompression for severe GO, the differences in GO-OF sensitivity toward certain stimuli reported here and in earlier studies underscore the importance of locally acting cytokines in controlling fibroblast activities in the affected orbital space in GO.

It is well recognized that numerous T-cell-, macrophage-, and connective tissue-derived cytokines may exert a variety of effects on fibroblasts including both stimulation or inhibition of extracellular matrix syn-
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FIGURE 2. Immunocytochemical staining for the Ki 67 nuclear antigen in orbital fibroblasts. Growth-arrested monolayers were treated for 72 hours as indicated and proliferating cells were detected by positive nuclear immunoreactivity for the proliferation-associated Ki 67 antigen (arrows). (A) Growth-arrested, untreated GO orbital fibroblasts; (B) IL-1α (10 U/ml)-treated GO orbital fibroblasts; (C) IL-1α (10 U/ml)-treated normal orbital fibroblasts; (D) PDGF-treated (1 ng/ml) GO-orbital fibroblasts (original magnifications X 240).

thesis, cytokine production, and the synthesis of certain cellular proteins. Retroocular fibroblasts are more sensitive to some of these effects than are fibroblasts derived from other anatomic sites. We demonstrated previously that retroocular fibroblasts are particularly sensitive to the glycosaminoglycan-stimulating effect of interferon gamma, but that fibroblasts from several other sites respond equally to IL-1α and TGFβ in terms of glycosaminoglycan production. In addition, we showed that interferon gamma and TNFα can induce or enhance the expression of HLA-DR molecules in these cells, an effect found to be of greater magnitude in retroocular fibroblasts derived from patients with GO than in those derived from normal persons. Further, several lines of evidence suggest that site-specific differences exist with respect to the metabolic responses of fibroblasts to certain stimuli including triiodothyronine and glucocorticoid agonists. Recently, we demonstrated that interferon gamma, TNFα, IL-1α, interleukin-6, TGFβ, and various other agents are capable of stimulating the expression of a 72 kD heat shock protein to a greater extent in retroocular fibroblast monolayers derived from patients with GO than in those from normal persons.

Stimulation of immunologically relevant molecules such as HLA-DR, adhesion molecules, and heat shock proteins on orbital fibroblasts by cytokines in GO may be important in the communication of connective tissue cells, immunocompetent cells, and extracellular matrix components. Additionally, these autocrine and paracrine factors are capable of modulating glycosaminoglycan synthesis and cell proliferation in orbital fibroblasts. These mechanisms may lead to the increase in orbital tissue volume and the impairment of extraocular muscle function that are responsible for the mechanical complications in GO. Inhibition of cytokine-mediated OF proliferation by glucocorticoid agonists, an effect that appears to involve the glucocorticoid receptor, may be one mechanism by
FIGURE 3. Modulation of IL-1α-stimulated orbital fibroblast proliferation by neutralizing anti-cytokine antibodies. Fibroblast monolayers, derived from orbital fatty connective tissue of patients with severe GO, were growth-arrested and coincubated with IL-1α and antibodies for 72 hours, as indicated. The proportions of proliferating cells were determined immunocytochemically, using an anti-Ki 67 monoclonal antibody that recognizes the Ki 67 nuclear antigen. Data represent the means ± SD of triplicate experiments.

which glucocorticoids exert their beneficial effects in GO.

Key Words
oribital fibroblast, Graves' ophthalmopathy, cell proliferation, cytokines, glucocorticoid receptor agonist

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