Graves' ophthalmopathy—immunologic parameters related to corticosteroid therapy

Robert C. Sergott, Norman T. Felberg, Peter J. Savino, John J. Blizzard, and Norman J. Schatz

Patients with severe Graves' ophthalmopathy may or may not improve with systemic corticosteroids. In an attempt to find immunologic distinctions to correspond with this clinical phenomenon, we have evaluated various parameters in corticosteroid-responsive and corticosteroid-resistant individuals. Percentages and absolute numbers of thymus-derived active and total rosette-forming cells (A-RFC and T-RFC) underwent statistically significant (p < 0.001) increases during successful prednisone therapy in 17 patients. B lymphocytes and complement receptor mononuclear cells did not significantly change with steroid therapy. In the second group, five patients therapeutically resistant to corticosteroids presented with elevated A-RFC and normal T-RFC. When treated with oral prednisone, these patients' A-RFC decreased (p < 0.001), and the T-RFC were unchanged. Corticosteroids increased the lymphoblast transformation response to phytohemagglutinin (PHA) of a steroid-responsive patient, whereas steroids decreased the PHA lymphoblast transformation response of a corticosteroid-resistant patient. A disease activity index to correlate the clinical and immunologic data has been devised. The findings may allow the clinician to predict which patients with Graves' ophthalmopathy are likely to benefit from corticosteroid therapy and which patients may be managed better by other methods.

Key words: Graves' disease, thyroid ophthalmopathy, erythrocyte rosette-forming lymphocytes, lymphoblast transformation, B lymphocytes, erythrocyte complement receptor (EAC) rosettes, corticosteroids

Systemic corticosteroids have been the basis for treatment of immunologic disorders for many years. Despite extensive use, little knowledge exists concerning their mechanism of action in specific conditions. In regard to peripheral blood lymphocytes, most studies have focused upon the immunosuppressive action of these agents in normal individuals. 1-4 In many disorders it has been assumed that this immunodepressive action is responsible for their therapeutic effect. Several investigations, however, have described both immunodepressing and immunostimulating effects of corticosteroids upon different parts of the immune system. 5-7

Graves' ophthalmopathy with its orbital inflammation is an entity in which cortico-
Fig. 1. Characteristic injection over lateral rectus muscle.

Steroids have been used empirically. The part(s) of the immune system that they alter and the reason(s) that they may be beneficial to some, but not to all, patients with this disorder have remained elusive.

We have recently described a decrease in the percentages and absolute numbers of thymus-derived rosette-forming lymphocytes in the majority of patients with active progressive Graves' ophthalmopathy. We report here that patients with decreased T lymphocyte levels, as assayed by spontaneous active and total rosette-forming cells (A-RFC and T-RFC), respond most favorably to corticosteroids. This improvement is accompanied by a marked increase in percentages and absolute numbers of peripheral blood T lymphocytes as well as an enhanced lymphoblast transformation (LBT) response to phytohemagglutinin (PHA). Patients who clinically fail to respond to corticosteroids have initially elevated A-RFC but normal T-RFC. In addition, we have devised a disease activity index to correlate the patients' clinical improvement with alterations in their immunologic characteristics.

Methods

Patients. Seventeen corticosteroid-responsive patients (10 women and seven men; mean age 45.53 ± 14.82 years, range 29 to 80) with progressive Graves' ophthalmopathy were evaluated in a prospective clinical and immunologic study from October 1976 to April 1979. Nine patients had painful, restrictive ophthalmoplegia, proptosis in excess of 23 mm, and corneal epithelial breakdown (Werner class 4 to 5). Eight patients had the previously mentioned clinical changes plus decreased visual acuity secondary to dysthyroid optic neuropathy (Werner class 6).

All patients were clinically and biochemically euthyroid when corticosteroid therapy was instituted. Five of 17 patients had one episode of Graves' thyrotoxicosis successfully treated with propylthiouracil (200 mg four times daily) at least 2 months prior to the onset of progressive ophthalmopathy. Six patients had received radioactive iodine 6 to 18 months prior to the development of ophthalmopathy. By clinical history and laboratory evaluation, five patients never had an episode of thyrotoxicosis but had a nonsuppressible pituitary-thyroid axis by either the Werner suppression test or an intravenous thyrotropin-releasing hormone (TRH) stimulation test. One patient presented with hypothyroidism without prior history of thyrotoxicosis.

Five corticosteroid-resistant patients (three women and two men; mean age 49.60 ± 15.36 years, range 25 to 63) were also studied. Three patients were in class 4 to 5, and two were in class 6. Similar to the corticosteroid-responsive individuals, these patients were clinically and biochemically euthyroid before prednisone therapy was begun. Three corticosteroid-resistant patients
were maintained on propylthiouracil (100 mg four times daily), one patient had received radioactive iodine 12 months prior to the development of ophthalmopathy, and one patient had euthyroid Graves' ophthalmopathy without prior history of thyrotoxicosis or hypothyroidism. No patient in either the corticosteroid-responsive or the corticosteroid-resistant groups had thyroid surgery.

**Disease activity index.** Since thyroid eye disease is well known for the unpredictable course of remissions and exacerbations, it was essential to devise an index of clinical disease activity to correlate with immunologic parameters.

The five clinical determinations found to be the best indicators of active, progressive ophthalmopathy in Werner's class 4 to 5 were (1) injection over the horizontal extraocular muscles; (2) pain with ocular motility, especially on attempted upgaze; (3) increased resistance to retropulsion of the globes; and for patients in class 6 (4) decreased Snellen visual acuity and (5) acquired dyschromatopsia documented by Ishihara pseudoisochromatic color plates.

Injection over the extraocular muscles was graded from 0 to 4+. This injection is characterized by a violaceous color with dilated blood vessels over the horizontal extraocular muscles (Fig. 1). Pain with attempted upgaze was graded from 0 (no pain) to 4+ (inability to maintain upgaze for more than 3 sec). Patients often described a sensation of "pulling" of their eyes while looking up. This painful upgaze was one of the first parameters to improve with corticosteroid therapy and one of the first to return with disease reaction.

Resistance to retrodisplacement of the globes into the orbit by gentle digital ballotment was rated between 0 (retrodisplacement in normal controls) and 4+ (no retropulsion). Similar to the pain with ocular movement, retrodisplacement often produced discomfort. Again, this finding responded promptly to corticosteroids and returned quickly with disease exacerbation.

Best corrected visual acuity of 6/9 or less in one eye in a patient without a prior history of impaired vision or a decrease in visual acuity of two lines on the Snellen chart was given a score of 4+. There was no attempt to grade loss of visual acuity from 0 to 4+—a patient either maintained or lost vision. Ability to identify less than 4 of 14 Ishihara color plates with each eye was graded 4+; identifying 4 to 7 was graded 3+; identifying 8 to 10 was graded 2+; identifying 9 to 11 was graded 1+, and identifying 12 or more was considered to be normal and graded 0. No patient had a history of hereditary or acquired defective color vision. Loss of color vision was often noticed by patients to precede loss of visual acuity. Patients with decreased visual acuity secondary to corneal exposure retained the ability to identify color plates correctly, unlike those with optic nerve disease. The clinical index is summarized in Table I.

**Table I.** Parameters in disease activity index

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rating</th>
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<tbody>
<tr>
<td>Visual acuity:</td>
<td></td>
</tr>
<tr>
<td>6/9 or better</td>
<td>0</td>
</tr>
<tr>
<td>6/9 or loss of two lines</td>
<td>+4</td>
</tr>
<tr>
<td>Ability to identify Ishihara color plates:</td>
<td></td>
</tr>
<tr>
<td>total 14</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>9-11</td>
<td>+1</td>
</tr>
<tr>
<td>8-10</td>
<td>+2</td>
</tr>
<tr>
<td>4-7</td>
<td>+3</td>
</tr>
<tr>
<td>4</td>
<td>+4</td>
</tr>
<tr>
<td>Injection over horizontal extraocular muscles</td>
<td>0-4+</td>
</tr>
<tr>
<td>Pain with motility</td>
<td>0-4+</td>
</tr>
<tr>
<td>Resistance to retrodisplacement of globes by digital ballotment</td>
<td>0-4+</td>
</tr>
</tbody>
</table>

Patients in class 4 to 5 with the most active clinical disease could have a maximum score of 12 because they do not have optic neuropathy.

Patients in class 6 could have a maximum score of 20 because each class usually, but not necessarily, includes the involvements indicated in the preceding class. Once visual loss occurs from dysthyroid optic neuropathy, patients are in class 6.

The clinical disease activity index was determined in each patient (by R. C. S. and P. J. S.) without knowledge of the immunologic data. Decision to modify corticosteroid therapy was made without knowledge of immunologic data and based on the clinical response alone.

**Endocrinologic evaluation.** All patients underwent a complete medical and endocrinologic examination (by J. J. B.). All patients had thyroxine, triiodothyronine, and T3 resin uptake percentages determined by conventional laboratory methods.

**Erythrocyte rosette-forming cells.** For active and total rosette determinations, 0.10 ml of the mononuclear leukocyte suspension at 5 × 10⁶ cells/ml (prepared by Ficoll-Hypaque gradient centrifugation) was mixed with 0.02 ml of absorbed normal human serum and 0.10 ml of 0.5% sheep erythrocytes. Ficoll-Hypaque centrifugation yielded a mononuclear population with over 95% lymphocytes. Viability, assessed by trypan
Table II. Percentages* of A-RFC and T-RFC in corticosteroid-responsive and corticosteroid-resistant patients with Graves’ ophthalmopathy

<table>
<thead>
<tr>
<th>Group</th>
<th>% A-RFC (No. of patients)</th>
<th>% T-RFC (No. of patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroid-responsive:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>13.71 ± 9.80 (17)</td>
<td>34.53 ± 12.10 (17)</td>
</tr>
<tr>
<td>During treatment</td>
<td>31.18 ± 10.44 (17)*</td>
<td>58.89 ± 8.70 (17)*</td>
</tr>
<tr>
<td>Corticosteroid-resistant:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>36.00 ± 7.81 (5)</td>
<td>48.60 ± 10.36 (5)</td>
</tr>
<tr>
<td>During treatment</td>
<td>17.40 ± 7.70 (5)*</td>
<td>46.00 ± 7.91 (5)t</td>
</tr>
<tr>
<td>Controls</td>
<td>24.78 ± 10.66 (100)</td>
<td>55.89 ± 10.75 (100)</td>
</tr>
</tbody>
</table>

*Results reported here and in Table IV are mean percentages ± standard deviation of the mean.

Table III. Absolute numbers* of A-RFC and T-RFC in corticosteroid-responsive patients (cells/mm³)

<table>
<thead>
<tr>
<th>Before therapy</th>
<th>During therapy</th>
</tr>
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<tbody>
<tr>
<td>A-RFC</td>
<td>258.43 ± 221.01 (13)</td>
</tr>
<tr>
<td>T-RFC</td>
<td>676.00 ± 311.66 (13)</td>
</tr>
</tbody>
</table>

*p value, before vs. during therapy = not significant.

Table IV. Percentages of B and EAC cells in corticosteroid-responsive patients

<table>
<thead>
<tr>
<th>Before therapy</th>
<th>During therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>9.88 ± 5.49 (8)</td>
</tr>
<tr>
<td>EAC</td>
<td>32.00 ± 14.85 (8)</td>
</tr>
</tbody>
</table>

No. of patients in parentheses.

Table V. Absolute numbers of B-cells in corticosteroid-responsive patients

<table>
<thead>
<tr>
<th>Before therapy</th>
<th>During therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>176.60 ± 100.58 (8)</td>
</tr>
</tbody>
</table>

No. of patients in parentheses.

There are no significant differences in the absolute numbers of B-cells before and during therapy.

blue exclusion test, was over 95%. After 5 min of 37°, the sample was centrifuged at 200 × g at room temperature for 5 min. Resuspended cells were examined in a hemocytometer for A-RFC. 13 T-RFC were determined in replicate specimens after an additional incubation for 90 min or overnight at 4°.

White blood cell and differential counts. White blood cell counts (cells/mm³) were determined in a Fisher autocytometer II. White blood cell differential counts were determined by counting 100 cells on Wright-stained peripheral blood smears.

LBT. LBT was performed according to the method of Oppenheim and Schecter. 14 Mononuclear leukocytes were prepared by Ficoll-Hyapque centrifugation and washed twice in RPMI-1640 medium containing 2 mm of glutamine, 10% normal human serum (heat-inactivated) (GIBCO), and 50 µg/ml gentamicin (Schering Corp.). Lymphocytes were resuspended at 5 × 10⁶ cells/ml, and 25 µl aliquots were added to wells of a flat-bottom microtitration tissue culture plate (Linbro). Various dilutions of PHA (Burroughs-Wellcome), concanavalin A (Con A; Pharmacia), and pokeweed mitogen (PWM; GIBCO) were added to different wells, and the wells were diluted to a final volume of 200 µl. PHA was used at a 1:32 dilution of the concentration supplied by the manufacturer. Con A was used at a final concentration of 160 µg/ml, and PWM was used at a 1:30 dilution of the concentration supplied by the manufacturer. After 72 hr of incubation, 1.0 µCi of tritiated thymidine was added to each well. After an additional 16 hr incubation at 37°, wells were collected in a MASH II harvester (Microbiological Associates). Collected cells were counted for radioactive incorporation in a liquid scintillation counter, and the results as counts per minute for each mitogen concentration (done in quadruplicate) were compared to the unstimulated control by the Student’s t test.

Complement receptor (EAC) rosettes. EAC rosettes were determined by modification of the method of Winchester and Ross. 15 Sheep erythrocytes were coated with subagglutinating dilutions of hemolysin followed by subhemolytic dilutions of guinea pig complement (EAC). A 100 µl volume of EAC (0.5%) was incubated with an equal volume of mononuclear leukocytes at 5 × 10⁶/ml. After
rocking 5 min at room temperature, the cells were centrifuged at low speed, gently resuspended, and enumerated in a manual hemocytometer. Rosettes were considered as any lymphocyte containing three or more EAC on its surface.

**B cell rosettes (Immunobeads).** B cells were enumerated in patients' mononuclear leukocyte populations by commercially available Immunobeads according to the supplier's instructions (Bio-Rad Laboratories). Beads containing rabbit anti-human immunoglobulins (heavy- and light-chain specific) were resuspended at 0.5% in Hanks' balanced salt solution, and 100 μl aliquots mixed with an equal volume of mononuclear leukocytes at 5 × 10⁶ cells/ml. After mixing, the cells were allowed to incubate at 4°C overnight. A 100 μl volume of 0.03% toluidine blue was added, and the cell button was gently resuspended. Cells were counted in a hemocytometer. An Immunobead (B cell) rosette was considered as any lymphocyte containing three or more Immunobeads on its surface.

**Results**

**Peripheral blood lymphocytes.** Table II shows the percentages of A-RFC and T-RFC in steroid-responsive and steroid-resistant patients. Table III shows the absolute numbers of A-RFC and T-RFC in steroid-responsive patients. After the institution of oral prednisone therapy, steroid-responsive individuals underwent statistically significant increases (p < 0.001, by the Student's t test) of both the percentages and absolute numbers of A-RFC and T-RFC. In contrast,
Fig. 4. Clinical course of corticosteroid-responsive patient showing the disease activity index, prednisone dosage, and percentages of rosette-forming cells. Shaded area, Period of alternate-day prednisone therapy; T, average and 1 S.D. for T-RFC in 100 normal controls; A, average and 1 S.D. for A-RFC.

Corticosteroid-nonresponsive patients underwent a statistically significant decrease (p < 0.001) in percentages of A-RFC. Percentages of T-RFC were not significantly altered in corticosteroid-resistant individuals. Control values in an age- and sex-matched population of 100 euthyroid individuals without known immunologic disease are also reported in Table II.

Table IV indicates that there was no statistically significant change in the percentages of B and EAC cells in corticosteroid-responsive patients. In addition, the absolute number of B cells was not altered by corticosteroids (Table V). B and EAC cells were determined in only two of the corticosteroid-resistant patients and did not show any statistically significant change when values before and during therapy were compared.

LBT. In one corticosteroid-responsive patient and in one corticosteroid-resistant patient LBT response to three mitogens was tested.

Fig. 2 illustrates the LBT for a patient responsive to corticosteroid therapy. When the patient was treated with prednisone, the LBT response to PHA increased over the pre-treatment values. In contrast, Fig. 3 shows the LBT of a patient resistant to corticosteroids. This patient's thyrotoxicosis was controlled with propylthiouracil, but the ophthalmopathy worsened, and LBT response to the various mitogens was essentially unchanged. Corticosteroid therapy was begun, LBT decreased, and the patient's ophthalmopathy worsened. Hence, compared to the patient in Fig. 2, corticosteroids caused a completely opposite clinical and immunologic response.
**Disease activity.** In three patients the disease activity index was correlated with erythrocyte rosette-forming cells and prednisone treatment. In Fig. 4 the percentage of erythrocyte rosette-forming cells rose from initial values of 2% and 38% to levels of 30% and 55% as the disease activity index fell from 12 to 0. When the prednisone was decreased to 40 mg daily, the disease activity index rose, and the rosette-forming cells decreased to 20% and 38%. When corticosteroids were again increased, the rosette-forming cells increased, whereas the disease activity index declined. The shaded area illustrates a trial period of alternate-day steroid therapy. The patient was then returned to daily therapy, and her disease went into remission. After prolonged corticosteroid therapy, rosette-forming cell enumeration was not as accurate a guide to disease activity as were the results obtained after the initiation of treatment. This lack of correlation with long-term corticosteroid therapy has been seen in several other patients.

Fig. 5 illustrates another patient who improved with corticosteroids. This patient initially had normal percentages of A-RFC. After 4 days of therapy, the patient had improved clinically, the percentage of T-RFC increased, and the absolute number of both A-RFC and T-RFC increased. This increase is in direct contrast to the response of a steroid-resistant patient (Fig. 6). After this patient had received 80 mg of prednisone for 7 days, the A-RFC declined markedly, and the T-RFC rose slightly but not significantly. The patient’s disease activity index was unchanged. Steroids were discontinued after 4 weeks. The shaded area represents a 10-day course of orbital irradiation. During the irradiation treatment, the patient’s A-RFC increased but not the T-RFC. He did not improve clinically with irradiation therapy and eventually underwent orbital decompression surgery.

**Discussion**

Correlation of clinical and immunologic data has disclosed a rational method to treat some patients with Graves’ ophthalmopathy.

Our findings have implications specifically regarding Graves’ disease and, in a more general sense, raise some questions regarding the heterogeneity of human lymphocyte subpopulations. Previous reports concerning corticosteroids in Graves’ disease have been paradoxical. Patients have been described whose thyrotoxicosis resolved with corticosteroid treatment, whereas others developed thyrotoxicosis despite high-dose corticosteroid therapy.

For many years systemic corticosteroids have been an accepted treatment for severe Graves’ exophthalmos. Their usefulness, however, has been limited by several problems: (1) an inability to predict which patients will respond to corticosteroids, (2) a lack of understanding of their mechanism of action, and (3) a lack of clinical and immunologic guidelines to judge therapeutic effectiveness.
so as to enable the clinician to maximize benefits and minimize complications.

Our finding of a depression in A-RFC and T-RFC percentages and absolute numbers in many patients with progressive ophthalmopathy9 offered an immunologic parameter to correlate with a patient's clinical status. We have shown in 17 patients that this depression of spontaneous rosette-forming thymus-derived lymphocytes undergoes a statistically significant increase to normal and supranormal levels with corticosteroid therapy and that the increase initially parallels the patients' clinical improvement (Tables II and III, and Figs. 4 and 5). B lymphocytes and EAC cells were not altered with corticosteroid treatment (Tables IV and V).

In patients improved with prednisone therapy, we found that the spontaneous rosette-forming cells increased within 4 to 5 days (Figs. 4 and 5). Conversely, if patients had initially elevated A-RFC and normal T-RFC, they failed to improve although their A-RFC decreased and T-RFC were unaltered with corticosteroid treatment (Table II and Fig. 6). With prolonged therapy, however, A-RFC and T-RFC failed to correlate precisely with clinical states—probably because of the diverse functional heterogeneity of the lymphocyte populations comprising A-RFC and T-RFC. When the patients' disease went into remission and prednisone was tapered, A-RFC and T-RFC remained elevated—approaching levels previously reported by us to be found in patients with stable, nonprogressive class 4 to 5 disease.9 Thus, in the 17 patients successfully treated with corticosteroids, we had an immunologic parameter which we could initially combine with the patient's clinical response to titrate the corticosteroid regimen. In contrast, for the five patients unresponsive to corticosteroids, we were able to move quickly to an alternative form of treatment such as supervoltage radiation therapy18 or surgical orbital decompression.19

Presently it appears necessary to examine both the initial level of T lymphocytes and their subsequent increase with prednisone. This dual analysis is essential because determinations of A-RFC and T-RFC percentages and absolute numbers are by no means immunologically specific assays for Graves' ophthalmopathy. Some relatively common conditions such as viral upper respiratory tract infections20 may also alter T lymphocyte numbers. Even though our study population was not affected, it is certainly conceivable that a viral infection in a patient with Graves' disease could cause depressed T lymphocyte values that would be unrelated to the ophthalmic manifestations of the disorder. Hence, in this case, the virally reduced T lymphocyte levels would be a false-positive test for corticosteroid-responsive Graves' ophthalmopathy. Presumably prednisone would fail to alter the ophthalmopathy and would also fail to increase the T lymphocyte levels.

Two corresponding clinical and immunologic groups of Graves' ophthalmopathy patients have evolved from this investigation—corticosteroid-responsive and corticosteroid-resistant. In the first instance, A-RFC and T-RFC as well as LBT increased, and the patient improved (Fig. 2). But in apparently the same clinical situation, corticosteroids had an opposite effect, since A-RFC and LBT decreased and the ophthalmopathy worsened. The clinical findings and corticosteroid therapy was precisely the same in each case. Only the immunologic data differed. Hence this immunologic dichotomy offers two feasible explanations. (1) There may be different immunologic mechanisms to reach the final common expression of the clinical entity of Graves' ophthalmopathy, or (2) there may be corticosteroid-resistant and corticosteroid-responsive phases of the same disorder.

Kriss and Mehdi21 have provided in vitro data that two mechanisms for Graves' ophthalmopathy may exist. With a vesicle containing eye muscle membrane protein, they have shown that there are two different in vitro pathways for lymphocytes from Graves'
disease patients to cause cell-mediated vesicle lysis. Their finding also suggests that all Graves' ophthalmopathy may not be pathogenetically the same entity.

Our study also has implications regarding human lymphocyte subpopulations. To our knowledge these data are the first report regarding a human disease state in which corticosteroids may not necessarily be immunodepressing. In fact, the increase in spontaneous rosette-forming thymus-derived lymphocytes and possibly the increased LBT response to PHA observed in one patient suggests that as measured by certain parameters, in specific diseases corticosteroids may be immunostimulating—or at least immunostabilizing. More patients will have to be serially evaluated to confirm the initial LBT data. The belief that corticosteroids decrease T lymphocyte percentages and absolute numbers is based upon studies in normal volunteers and in some specific diseases.1-4 In view of our findings, it is improper to extrapolate these results to other disorders. The varied effects of corticosteroids upon human lymphocyte subpopulations is not unexpected, since in animals there is strong evidence for varying responses of T cells to corticosteroids.22-24

There are several possible mechanisms for the corticosteroid-induced T lymphocyte increase: (1) altering the lymphocyte membrane to allow the cells to bind to sheep red blood cells; (2) changing the peripheral circulation and distribution of lymphocytes by causing an outpouring of T cells from various storage sites such as thymus or spleen; (3) preventing the egress of cells from the peripheral circulation to sites of tissue damage such as extracellular muscle or orbital fat; or (4) allowing the maturation of T cells from T cell precursors such as the "null" cells, either by a direct effect on immature T cells or by lysing a subpopulation of cells that block T cell maturation.

Elucidating the mechanism of the prednisone-induced T cell increase should enable Graves' ophthalmopathy to be treated with more immunopharmacologic precision. The final goal is to allow the clinician to predict which patients will improve with corticosteroids and which patients should be treated primarily with other modalities.

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REFERENCES


