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Immunohistochemical Analysis of Iris Biopsy Specimens From Patients With Fuchs' Heterochromic Cyclitis

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Using immunohistochemical techniques, we analyzed iris biopsy specimens from eight patients with Fuchs' heterochromic cyclitis, seven patients with various other types of uveitis, and eight glaucoma patients without uveitis. No specific abnormalities related to Fuchs' heterochromic cyclitis could be detected. Four of the patients with Fuchs' heterochromic cyclitis and four of the patients with uveitis showed evidence of an inflammatory cell infiltrate, which was a mixture of interleukin-2 receptor-negative T helper and suppressor cells, B lymphocytes, and plasma cells. Only an occasional T lymphocyte could be seen in two of the patients without uveitis. The class II antigen HLA-DR was expressed on iris stromal cells in every patient in the Fuchs' heterochromic cyclitis group and uveitis group and in six of the patients in the nonuveitis group. In six of the Fuchs' heterochromic cyclitis patients, including two without immunohistochemical evidence of inflammatory cell infiltrate, histologic abnormalities were present on hematoxylin and eosin sections.

VARIOUS HYPOTHESES have been proposed about the pathogenesis of Fuchs' heterochromic

cyclitis.¹ Recent evidence suggests, however, that disturbances of the immunoregulatory system play an important role.²⁻⁴ Quantitative examination of peripheral blood lymphocytes in Fuchs' heterochromic cyclitis⁵ and other types of uveitis⁵⁻⁷ is generally unhelpful because immune events occurring focally within the eye may not be reflected in peripheral blood lymphocyte profiles. Examination of intraocular fluids and tissues is essential, therefore, to gain an understanding of pathogenic mechanisms. Although results of light and electron microscopic examination of the iris in Fuchs' heterochromic cyclitis are well documented,^{1,8-16} there have been no reports using immunohistochemical techniques. These techniques have been used, however, to study iris specimens obtained from patients with other chronic uveitis syndromes,¹⁷⁻²⁰ where T and B lymphocytes and increased expression of class II antigens have been found.

A slowly progressive B cell-mediated disease has been suggested as a pathogenic mechanism for Fuchs' heterochromic cyclitis¹ as a result of finding lymphocytes and plasma cells in the iris^{1,8-16} and reduced T suppressor cell activity in the peripheral blood.² To gain a greater understanding of the immunopathogenic mechanisms involved, we performed an immunohistologic examination of iris biopsy specimens from patients with Fuchs' heterochromic cyclitis. We compared the results with those in patients without uveitis and patients with other types of uveitis.

Material and Methods

Iris biopsy specimens (from peripheral iridec-
tomies) were obtained from eight patients
(seven men and one woman, aged 23 to 54
years) with Fuchs' heterochromic cyclitis and

Accepted for publication Jan. 8, 1990.

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seven patients (one man and six women, aged 59 to 77 years) with various other types of uveitis, classified according to the recommendations of the International Uveitis Study Group.²¹ Diagnoses included idiopathic chronic anterior uveitis (three patients), panuveitis (three patients, two of whom had proven sarcoidosis), and idiopathic (HLA-B27-negative) recurrent anterior uveitis (one patient). Specimens were obtained either during extracapsular cataract extraction or trabeculectomy. At the time of surgery, no patients with Fuchs' heterochromic cyclitis were receiving systemic treatment, but two were using topical antiglaucoma preparations and two were using topical corticosteroid preparations. Two patients with uveitis were receiving systemic corticosteroids, four were using topical antiglaucoma preparations, and three were using topical corticosteroid preparations. Iris specimens were also obtained from eight patients without uveitis (two men and six women, aged 40 to 85 years) who had chronic simple glaucoma and were undergoing trabeculectomy. No patient received systemic therapy, and all patients were using topical antiglaucoma medication. Four of the patients had undergone argon laser trabeculoplasty in the year before surgery. We chose glaucoma patients, rather than patients undergoing extracapsular cataract extraction and intraocular lens implantation for senile cataract, for the group without uveitis because peripheral iridectomy is an integral part of trabeculectomy. All patients were informed about these investigations and their consent was obtained.

Specimens were snap-frozen in optimal cutting tissue compound within one hour of iridectomy. Cryostat sections of 6- μ m thickness were air dried, fixed in acetone for ten minutes, and rinsed in phosphate-buffered saline, pH 7.4. Slides were then incubated for one hour at room temperature with a panel of monoclonal antibodies in phosphate-buffered saline containing 0.01% gelatin and 0.1% sodium azide. The antibodies used were as follows: as a pan T cell marker, we used antibodies against the CD2 (Dakopatts) and CD7 (Cell Biology Laboratory, Rotterdam) antigens. For the T helper and T suppressor/cytotoxic subset, antibodies against CD4 and CD8 markers were used (Becton Dickinson). Antibodies against the CD19 and CD20 antigens were applied to stain the B cells (Coulter Clone). Plasma cells were identified using separate antibodies against the immunoglobulin kappa and lambda light chains (Cell Biology Laboratory). Other mark-

ers included antibodies against major histocompatibility complex class II antigens (Becton Dickinson) and the interleukin-2 receptor.

After rinsing in phosphate-buffered saline, slides were incubated for one hour at room temperature with peroxidase-conjugated polyclonal rabbit anti-mouse immunoglobulins (Dakopatts, Copenhagen, Denmark). Subsequently, slides were thoroughly washed in phosphate-buffered saline, and antigen-antibody binding was visualized using the substrate 3-amino-9-ethylcarbazole.²² Sections were counterstained with Mayer's hematoxylin to obtain a discrete nuclear staining pattern. Positive reactions produced a red color, which made differentiation from dark brown endogenous melanin possible. Replacement of the primary antibody by phosphate-buffered saline served as a negative control. One slide from each specimen was stained with hematoxylin and eosin to evaluate the following pathologic features: focal depigmentation of iris pigment epithelium, hyalinization of vessel walls, iris stromal atrophy and fibrosis, and the disappearance of stromal melanocytes. One slide of each specimen was also stained with toluidine blue to detect mast cells.

Tissue sections were examined using 25 $\times$ magnification by two independent observers (P.I.M. and C.M.M.), both of whom were unaware of the diagnosis. The scoring system described in the legend of Figure 1 was used.

Results

Immunohistologic findings for each group are summarized in Figure 1. No correlation could be seen between those patients receiving systemic or topical corticosteroid therapy, or both, and the presence or absence of any specific cell type.

In the Fuchs' heterochromic cyclitis group, four patients had a stromal infiltrate of CD2- or CD7-positive cells, or both, and of these there appeared to be an equal distribution of CD4- and CD8-positive cells (Fig. 2). Staining for the interleukin-2 receptor was negative for each patient. Although two patients showed scattered and clustered CD19- or CD20-positive cells, or both, in the iris stroma, kappa/lambda immunoglobulin G light chain staining on plasma cells was seen in three patients. This latter finding appeared to correlate well with the number of plasma cells seen on the correspond-

Fig. 1 (Murray and associates). Pan T cells (CD2/7 [squares]), B cells (CD19/20 [triangles]), and HLA-DR-positive cells (circles) in iris tissue from eight patients with Fuchs' heterochromic cyclitis, seven patients with uveitis, and eight patients with nonuveitic glaucoma (control patients). The following scoring system, which assessed positive staining cells from two sections, was used: 0 indicated zero to one cell; 1, few cells; 2, cells scattered or in small clusters; and 3, cells scattered and in clusters.

ing hematoxylin and eosin sections. Every patient had expression of the class II antigen HLA-DR, which was present on iris resident cells throughout the stroma, around the vessels, in the iris sphincter, and in the anterior cell border layer. Of the four patients without T or B lymphocyte infiltration, two showed histologically some evidence of focal depigmentation of the iris pigment epithelium, marked hyalinization of vessel walls, and iris stromal atrophy and fibrosis. Mast cells and a reduction in the number of stromal melanocytes were each found in three patients.

The findings in the patients with uveitis were similar to those in patients with Fuchs' heterochromic cyclitis. T and B cell markers were seen in four and two patients, respectively. HLA-DR-positive cells were also seen in every patient, and no patient was positive for the interleukin-2 receptor.

In the group of patients with glaucoma but not uveitis, only a few T cells, which were interleukin-2 receptor-negative, were seen in two patients. No B cells were identified. HLA-DR was expressed in six patients on single cells scattered throughout the stroma and in fewer numbers than in the other groups.

Discussion

No specific immunohistologic abnormalities in the irises of patients with Fuchs' heterochromic cyclitis were identified. We found no relationship between the various positive results in the same individual. The uveitis group also showed similar nonspecific staining results, which corresponds with those found in previous studies.¹⁷⁻²⁰

Fuchs' heterochromic cyclitis has previously been described as an "intraocular B cell factory."¹ Many light and electron microscopic studies^{1,8-16} that demonstrate a lymphocyte and plasma cell infiltrate in the iris support this description. In this study, however, a lymphocytic infiltrate was present in only one half of the patients examined, and plasma cells were present in three of eight patients. B lymphocytes were seen in only two of the three patients with plasma cells. This discrepancy can be explained because CD19 and CD20 molecules are expressed during the stages of B lymphocyte maturation but not on plasma cells.²³

Despite the lack of immunohistologic evidence of T and B lymphocytes and plasma cells, characteristic findings of Fuchs' heterochromic cyclitis were present on hematoxylin and eosin sections in two patients. In one of these patients, local immunoglobulin G production and aqueous oligoclonal immunoglobulin G bands were demonstrated by isoelectric focusing and immunoblotting (unpublished data). This implies that there are clones of plasma cells present that were not detected by the methods used in this study, for which there may be two reasons; the small biopsy specimens obtained by peripheral iridectomy may not be representative of the whole iris, or immunoglobulin G is produced by plasma cells elsewhere in the eye.

Previous studies have demonstrated focal depigmentation of iris pigment epithelium, hyalinization of vessel walls, stromal atrophy and fibrosis, and reduced numbers of stromal melanocytes in the iris. Not all of these findings have been present, however, in every patient. In this study, evaluation of the possible loss of pigmented cells from the iris stroma was considered unreliable because we did not have a specimen of the opposite, normal iris for com-

Fig. 2 (Murray and associates). Iridectomy specimen from a patient with Fuchs' heterochromic cyclitis (Patient 2). Top left, Clustered T cell (CD2-positive) infiltrate ($\times 200$). Top right, Higher magnification of a T cell (CD2-positive) cluster ($\times 450$). Bottom left, Scattered B cell (CD19-positive) infiltrate ($\times 200$). Bottom right, Higher magnification of this scattered B cell (CD19-positive) infiltrate ($\times 450$).

parison. Any loss, thinning, or irregularity of the iris pigment layer could have been attributable to artifact or surgical trauma. Also, hyalinization of vessel walls is known to occur to some degree in normal irises. Similarly, the demonstration of lymphocytes and plasma cells is a characteristic but not consistent finding in Fuchs' heterochromic cyclitis; a previous study¹⁶ also failed to detect these cell types in

some of their patients. It may be that the histologic appearance depends on the chronicity of the disease, and those patients who have had the disease for longer will show more abnormalities. This appears not to be the case in this study, because the two patients who failed to show any abnormality on immunohistochemical and hematoxylin and eosin staining had their condition diagnosed many years earlier

than some patients with characteristic histologic abnormalities. This inconsistency may also be attributable to the size of the specimen examined, because most of the previous histologic studies^{8-12,14,15} were performed on whole globes and on specimens from sector iridectomies rather peripheral iridectomies.

The enhanced expression of HLA-DR on iris resident cells in the Fuchs' heterochromic cyclitis and uveitis groups may indicate a state of immunologic responsiveness,²⁴ but the T lymphocytes seen in the four patients in each of these groups all appeared to be nonactivated because they were interleukin-2 receptor-negative. The class II antigen expression may be involved, therefore, in the perpetuation of the disease process. Mast cells may also play a role in the pathogenesis of some types of uveitis,²⁵ but the increased number of mast cells previously reported in Fuchs' heterochromic cyclitis¹⁶ could not be confirmed.

The absence of any notable lymphocytic infiltrate and the presence of HLA-DR in the glaucoma group without uveitis corresponds to findings in previous studies,^{18,19} but the exact figures for those patients with chronic simple glaucoma were not given. Expression of class II antigens in the normal iris has also been reported by others,²⁶ although some have refuted this observation.^{20,27,28} Previous work has shown a mild inflammatory reaction in the irises of animals that have undergone argon laser trabeculoplasty.²⁹ In this study, none of the four glaucoma patients without uveitis previously subjected to laser therapy showed any immunohistologic evidence of an inflammatory infiltrate.

Immunohistochemical analysis of iris biopsy specimens in Fuchs' heterochromic cyclitis failed to show any specific immunohistologic abnormality in what is a supposed uniform condition. Perhaps the classification of diseases based on clinical methods alone does not necessarily relate to the disease's pathologic findings. It is conceivable, therefore, that Fuchs' heterochromic cyclitis may be a single clinical entity with more than one histologic and pathologic mechanism.

ACKNOWLEDGMENTS

P. J. M. Bos, M.D., Amsterdam and P. Leonard, M.D., Leiden, provided some of the iridectomy specimens. The monoclonal antibodies against kappa and lambda light chains were donated by Theo van der Kwast, M.D., Rotterdam, The Netherlands.

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OPHTHALMIC MINIATURE

His uncle, on the other hand, developed a hoarse dry nervous cough and lapsed into almost total wordlessness. His eyes turned to pools behind the rimless glasses; the white and dark of the sockets blurred, became indistinguishable. Their sole light seemed to come from the occasional sudden flarings in the sun or in the fires of the furnace.

Chaim Potok, *The Book of Lights*
New York, Fawcett Crest, 1981, p. 5