Immunopathology of Chronic Experimental Histoplasmic Choroiditis in the Primate

Anthony Anderson,* William Clifford,* Ildiko Palvolgyi,* Lawrence Rife,* Clive Taylor,† and Ronald E. Smith*

A nonhuman primate model of ocular histoplasmosis was developed that enabled the authors to define the choroidal cellular immunopathology of both the acute and chronic phases of experimental histoplasmic choroiditis. Anti-human monoclonal antibodies were used to identify the inflammatory cell subsets and to calculate their relative percentages in the choroidal inflammatory lesions. Comparison of the acute (≤ 65 days) and chronic (≥ 1 yr) phases suggested possible variations in the evolution of these lesions, resulting in the development of immunopathologically distinct chronic lesions. In this model, these late lesions could be differentiated by the presence or absence of dense lymphocytic foci, comprised predominantly of mature B-lymphocytes, located within the more diffuse inflammatory cell background. The chronic lesions containing these B-cell foci had significantly higher percentages of both mature B-cells (P < 0.0001) and helper-inducer T-cells (P < 0.05) than did the chronic lesions without B-cell foci. The increase in helper-inducer T-cells in the chronic lesions with B-cell foci resulted in a higher mean helper-suppressor T-cell ratio (μ = 0.60) than that seen in lesions lacking foci (μ = 0.33). These findings suggest that, even in the same eye, individual chronic histoplasmic choroidal lesions, which clinically resemble “histo spots” in humans, may have different immunopotentials. Invest Ophthalmol Vis Sci 33:1637–1641, 1992

Although the pathogenesis of chronic ocular histoplasmosis is uncertain, one hypothesis postulates that chronic choroidal inflammation, involving sensitized lymphocytes, may be an important factor.1–6 We developed a nonhuman primate model of ocular histoplasmosis induced by intracarotid injection of live yeast-phase Histoplasma capsulatum. Using this model, we studied the immunopathology of this disease. The inflammatory cells in the choroidal lesions were characterized using hybridoma-derived monoclonal antibodies (MoAbs) that identify specific human leukocyte subsets. These MoAbs have been shown to exhibit cross-reactivity with the analogous cell type in the primate.7–9 We previously reported our preliminary findings on the immunopathology of the acute choroidal lesions.10 Herein, we describe the immunopathology of chronic lesions. By 1 yr after intracarotid injection of organisms, the acute lesions have evolved to resemble the inactive choroidal or chorioretinal scars (“histo spots”) seen in the human ocular disease. Comparisons of the immunopathologic findings from both the acute and chronic lesions are presented.

Materials and Methods

Animals

Histoplasmic choroiditis was produced in one eye of 13 Macaca mulatta or M. speciosa monkeys by means of a standardized intracarotid injection of live yeast-phase H. capsulatum. The surgical techniques and the pre- and postinjection clinical examination regimen have been reported previously.2–4,10,11 The care and maintenance of the animals and the performance of all procedures conformed to the ARVO Resolution on the Use of Animals in Research, and all animals were housed in facilities fully accredited by the American Association of Laboratory Animal Sciences.

A total of 40 acute (ie, < 65 days postinjection) choroidal lesions from six eyes and 25 chronic (ie, > 1 yr postinjection) choroidal lesions from seven eyes were studied. All animals were adults (age range, 5–15 yr) at the time of tissue collection.

Tissue Processing

Collection and cryostat sectioning of tissues and indirect immunoperoxidase staining techniques (using murine anti-human MoAbs for the immunopatho-
logic identification of choroidal inflammatory cells) have been reported. Tissues from acute lesions were collected 30–65 days after intracarotid injection, and the chronic lesions were collected 1–7 yr after injection.

Whenever possible, 85 or more slides of cryostat-sectioned tissue were made for each acute or chronic lesion to optimize sampling accuracy. The slides were numbered sequentially, and each slide was stained using an indirect immunoperoxidase technique and one of the MoAbs as the primary antibody. The staining sequence for the identification of inflammatory cell subsets was the following.

<table>
<thead>
<tr>
<th>Slide #</th>
<th>MoAb-identified cell subset</th>
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<tbody>
<tr>
<td>1</td>
<td>Suppressor/cytotoxic T cells (CD 8)</td>
</tr>
<tr>
<td>2</td>
<td>Immature B cells (IgD)</td>
</tr>
<tr>
<td>3</td>
<td>Mature B cells (CD 21)</td>
</tr>
<tr>
<td>4</td>
<td>Helper/inducer T cells (CD 4)</td>
</tr>
<tr>
<td>5</td>
<td>Immature B cells (IgD)</td>
</tr>
<tr>
<td>6</td>
<td>Mature B cells (CD 21)</td>
</tr>
<tr>
<td>7</td>
<td>Macrophages/polymorphonuclear leukocytes (OKM1)</td>
</tr>
</tbody>
</table>

This sequence was repeated throughout the entire set of slides obtained from each lesion. Additional slides from each cryosectioned lesion were used as control specimens, which consisted of either the omission of the primary antibody or the use of MoAbs of irrelevant specificity substituted for the primary antibody. After staining, all slides were photographed, as previously described.

Analysis

From each photograph, we counted the inflammatory cells that were stained (positive) for a specific MoAb, along with the counterstained (negative) background inflammatory cells. Our results are presented as the relative percentage of each inflammatory cell subset in a lesion. Statistical significance was determined using the Wilcoxon rank-sum test.

Results

A comparison of the cellular immunopathologic data from the acute and chronic histoplasmic lesions is presented in Table 1. Data on acute lesions, and the clinical and pathologic features of the natural history of this experimental disease, have been reported previously.

In the acute choroidal lesions, we frequently observed immunohistochemical staining of what appeared to be large numbers of macrophages. However, these macrophages could not be enumerated because of local necrosis and dense cellular packing. Therefore, the relative percentage of macrophages presented for the acute lesions may be artificially low.

As expected, chronic lesions contained markedly fewer total inflammatory cells than did acute choroidal lesions, and the cells were distributed somewhat more diffusely in the choroid. As seen in Table 1, suppressor–cytotoxic T-lymphocytes were the most abundant cells in both the acute and chronic lesions. In the acute lesions, helper–inducer T-lymphocytes were the second most frequent cells. By contrast, both

Table 1. Comparative cellular immunopathology of acute vs chronic (with and without B cell foci) histoplasmic choroidal lesions

<table>
<thead>
<tr>
<th></th>
<th>Acute* lesions (n = 40)‡</th>
<th>Chronic lesions† (n = 25)‡</th>
<th>Chronic lesions† with foci (n = 14)‡</th>
<th>Chronic lesions† without foci (n = 11)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helper/inducer T cells (CD4)</td>
<td>26.4 ± 9.7§</td>
<td>10.0 ± 7.2</td>
<td>13.3 ± 7.0</td>
<td>5.9 ± 4.3</td>
</tr>
<tr>
<td>Suppressor/cytotoxic T cells (CD8)</td>
<td>42.7 ± 8.6</td>
<td>27.3 ± 21.6</td>
<td>27.6 ± 16.7</td>
<td>26.9 ± 25.9</td>
</tr>
<tr>
<td>Immature B cells (IgD)</td>
<td>6.3 ± 7.0</td>
<td>2.9 ± 4.9</td>
<td>2.9 ± 2.9</td>
<td>2.9 ± 6.6</td>
</tr>
<tr>
<td>Mature B cells (CD 21)</td>
<td>5.5 ± 7.5</td>
<td>11.1 ± 17.1</td>
<td>19.3 ± 18.6</td>
<td>0.7 ± 0.9</td>
</tr>
<tr>
<td>Macrophages (OKM1)</td>
<td>7.1 ± 5.9</td>
<td>5.6 ± 7.3</td>
<td>3.2 ± 2.7</td>
<td>8.8 ± 9.4</td>
</tr>
<tr>
<td>Polymorphonuclear leukocytes</td>
<td>7.6 ± 5.9</td>
<td>15.6 ± 17.2</td>
<td>8.7 ± 7.1</td>
<td>24.5 ± 21.0</td>
</tr>
<tr>
<td>Helper/suppressor ratio</td>
<td>0.62 ± 0.19</td>
<td>0.48 ± 0.41</td>
<td>0.60 ± 0.47</td>
<td>0.33 ± 0.24</td>
</tr>
</tbody>
</table>

* Tissue collected 30–65 d after intracarotid injection of live *Histoplasma capsulatum*
† Tissue collected 1–7 yr after intracarotid injection of live *H. capsulatum*. Lesions differentiated by the presence or absence of lymphocytic foci made up primarily of mature B cells.

‡ Number of lesions studied.
§ Expressed as mean relative percent of total inflammatory cells ± standard deviation of mean.
¶ Significance between means determined by Wilcoxon’s rank sum test.
NS, not significant.
Fig. 1. Micrographs of tissue sections from a primate lesion of chronic ocular histoplasmosis. An indirect immunoperoxidase staining technique, employing anti-human monoclonal antibodies, was used to identify (red membrane staining) inflammatory cell subsets. (A) Stained to identify mature B (CD21+) lymphocytes. (B) Adjacent section stained to identify helper/inducer T (CD4+) lymphocytes. Note the lymphocytic foci within the background of scattered lymphocytes. Such foci, made up predominantly of mature B lymphocytes, were seen in lesions from 1–10 yr after intracarotid injection of live Histoplasma capsulatum. C, Choroid; R, Retina. Hematoxylin, ×355.

mature B-cells and polymorphonuclear leukocytes were found in greater relative percentages in the chronic lesions than were helper-inducer T-cells. These mature B-lymphocytes were not distributed randomly in the diffuse cell infiltrates comprising the chronic lesions but, rather, were almost invariably found in foci of densely compacted lymphocytes (Fig. 1). They were the most common cell type in these
foci, comprising more than 58% of the total cells. These foci were found in 14 of the 25 (56%) chronic lesions obtained 1 yr of more after intracarotid injection of organisms.

Because these B-cell foci appeared to be a distinct entity in the chronic lesions, we compared chronic lesions containing B-cell foci with chronic lesions without B-cell foci. In lesions containing foci, mature B-lymphocytes were the second most numerous cells, and they were present in a significantly (P < 0.0001, not shown in Table 1) higher percentage (19.3%) than in chronic lesions without foci (0.7%). The suppressor–cytotoxic cell percentages did not differ significantly; however, the helper–inducer T-cells were found in a significantly (P < 0.05, not shown in Table 1) higher relative percentage (13.3%) in chronic lesions containing foci than in chronic lesions lacking foci (5.9%). This resulted in a higher helper–suppressor T-cell ratio (0.60) in the scars with foci compared with those without foci (0.33).

Polymorphonuclear leukocytes were distributed randomly in all chronic lesions, but they were found in significantly higher (P < 0.05, not shown in Table 1) percentages in chronic lesions lacking B-cell foci than in chronic lesions containing these foci.

Because there were significant differences in some inflammatory cell percentages between the chronic lesions with and without B-cell foci, we compared these two types of chronic lesions to the acute lesions. The results (Table 1) provide some evidence for a differential development of chronic lesions in our primate model. It was observed that the relative percent of mature B-cells in the chronic lesions with B-cell foci was significantly higher (P < 0.0001) than in the acute lesions. By contrast, the relative percent of the mature B-cells in chronic lesions without B-cell foci was significantly lower (P < 0.001) than that in the acute lesions. When the relative percentages of mature B-cells in chronic lesions with and without B-cell foci were combined, they cancelled each other out statistically, and as seen in Table 1, superficially there appeared to be no significant difference in the relative percentages of mature B-cells in the acute versus chronic lesions. Other significant differences that provide additional evidence for the differential development of chronic lesions were seen in the macrophage and polymorphonuclear leukocyte populations and in the helper–suppressor ratios. It was determined that the macrophage population in the chronic lesions with B-cell foci was significantly lower (P < 0.05) than that in the acute lesions. Similarly, the polymorphonuclear leukocyte population was greater (P < 0.001) and the helper–suppressor ratio was lower (P < 0.01) in chronic lesions without B-cell foci than in the acute lesions. Again, none of these differences were evident when the acute lesions were compared with all chronic lesions, regardless of type.

Discussion

In our primate model, the chronic stage of the experimentally induced ocular histoplasmosis has a clinical course and histopathologic features similar to the human disease. The late lesions in our model resemble inactive choroidal scars or “histo spots,” but they often are accompanied by chronic inflammatory cell infiltrates and thus are not really “inactive.” Similar findings have been observed in human eyes with histoplasmosis.

Using this model, we studied the immunopathology of the experimentally induced disease. The use of anti-human MoAbs for the identification of inflammatory cell subsets in the choroidal lesions provided us with new insights into the underlying immunopathogenic mechanisms.

We previously reported that, during the acute phase of the disease, there was an infiltration of inflammatory cells into the choroid that produced the clinically visible lesions. The acute response to H. capsulatum involved large numbers of helper and suppressor T-lymphocytes and macrophages and small numbers of B-lymphocytes. By 1 yr after the initial infection, the lesions usually have resolved and resemble inactive scars. However, although the total cell infiltrate decreases markedly from that seen during the acute disease, some inflammatory cells persist; therefore, these scars are not truly inactive. In late lesions, T-lymphocytes represent the largest population of inflammatory cells, and there appears to be a marked increase in the proportion of mature (CD21) B-lymphocytes. These cells are found in foci of tightly compacted lymphocytes, comprised primarily of mature B-lymphocytes.

The identification of foci of mature B-lymphocytes in chronic ocular histoplasmic lesions (1 yr or more after intracarotid injection of H. capsulatum) was unexpected and raises questions about the origin of and stimulus for these cells. It is possible that they are remnants of the B-cell aggregates observed in the acute lesions. Alternatively, they could be recruited from the systemic pool of B-cells at a later time. In either case, such lymphoid cells would not be expected to remain in the choroid without an antigenic stimulus. Whether this stimulus is nondegraded, chitinous cell wall material of H. capsulatum, resulting from the acute choroidal disease, and/or other sequestered ocular antigens related to acute and chronic damage to the Bruch’s membrane and retinal pigment epithelium complex is unknown.

An interesting finding was that only approximately 50% of the chronic lesions contained foci of mature
B-lymphocytes. In humans with ocular histoplasmosis, and in animal models, not all late lesions reactivate. Therefore, the B-cell foci and a higher relative percentage of helper–inducer T-lymphocytes might be necessary for reactivation to occur. The presence of mature B-lymphocytes also suggests a role for antibody involvement in the chronic phase of ocular histoplasmosis. It is possible that not all chronic lesions have the same immunopotential for reactivation.

It is uncertain if the reactivation seen in human ocular histoplasmosis is driven immunologically. Our primate model provides some evidence that individual acute lesions may follow different natural history pathways, resulting in chronic lesions with different immunopotentials. However, it is possible that the cellular differences seen in the chronic lesions in our model reflect only different stages of the same evolutionary process. If this is true, it would suggest that individual lesions, even in the same eye, may evolve at different rates. We observed, for example, chronic lesions with and without B-cell foci as long as 10 yr after intracarotid injection of live *H. capsulatum*.

Although we did not see spontaneous reactivation of choroidal lesions in our primate model, this does not exclude the occurrence of this phenomenon over a more protracted time course and with a larger pool of infected animals. It has been proposed that ocular histoplasmosis is a disease that evolves over several decades. Therefore, the time frame over which we studied our model may not be sufficient to mimic the immunopathologic events associated with naturally occurring reactivation of this disease. We hope that additional studies will help to bridge the gaps in our understanding of the pathogenesis of the late macular manifestations of human ocular histoplasmosis.

**Key words:** chronic ocular histoplasmosis, nonhuman primate, choroid, immunopathology, monoclonal antibodies

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**References**