Mast Cells in Human Optic Nerve

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Purpose. Mast cells are classically found in ocular tissues within the conjunctiva, choroid, and iris. The aim of this study was to examine their distribution in the optic nerve and its meninges.

Methods. Sixty-six human optic nerves were studied from normal subjects at autopsy, fetuses aborted for chromosomal abnormalities, and from enucleation specimens of patients with a variety of inflammatory, traumatic, neoplastic, and vascular disorders. Mast cells were identified using a stain for the enzyme chloracetate esterase, and confirmed using toluidine blue, revealing metachromatic cytoplasmic granules.

Results. Mast cells were found scattered in the meninges of almost all optic nerves examined, frequently in perivascular locations, with densities up to 2325 mast cells/mm³ (mean 269.7 ± 64.1 cells/mm³ in normal nerves). Mast cells were found in the optic nerve parenchyma in nerves from four eyes that had severe abnormality, often associated with neovascularization. Normal nerves, as well as nerves from fetuses aborted for congenital defects, had significantly fewer meningeal mast cells than those from eyes with inflammatory or vascular diseases. De-granulation of mast cells was observed more often in eyes with recent severe trauma.

Conclusions. Based on their work and the work of others suggesting an association between mast cells and nervous system autoimmune disorders, the authors hypothesize a role for optic nerve mast cells in certain ocular inflammatory conditions. Invest Ophthalmol Vis Sci. 1993;34:3147-3153.

Mast cells are widely distributed, especially in connective tissue and mucosal surfaces. Although their role in normal physiological functioning is not known, they play a central role in allergic responses through the release of a number of inflammatory mediators. Mast cells can be stimulated both in an antigen-specific manner through binding to cell-surface immunoglobulin E, and nonimmunologically.1 In the eye, they are classically found in the conjunctiva, choroid, ciliary body, and iris; it has been theorized that they are involved in the pathophysiology of autoimmune uveitis.2 Workers in our laboratory have been studying the role of mast cells in experimental autoimmune demyelinating disease and have found that susceptibility to experimental autoimmune encephalomyelitis and experimental autoimmune neuritis correlate with the number of dural and sciatic nerve mast cells, respectively.3 Experimental autoimmune neuritis develops less readily in animals treated with the mast cell stabilizer nedocromil,4 and mast cells release proteases that can degrade myelin.5 As part of an understanding of the pathophysiology of events that may underlie inflammatory processes in the optic nerve, such as optic neuritis, we examined optic nerves for the presence of mast cells, using a stain for the enzyme chloracetate esterase, and with toluidine blue. We present here the first systematic study of the pattern and distribution of mast cells in human optic nerves from normal subjects, as well as subjects with a variety of ocular disorders.

METHODS

Sections from sixty-six human whole eyes previously submitted for pathological diagnosis after surgery or autopsy from subjects with either documented eye disease or no history of ocular pathology were studied (Table 1). The tenets of the Declaration of Helsinki were followed. All tissue used was obtained subsequent to pathological examination, and all patients undergoing enucleation had previously given in-
formed consent to the use of tissue that was not needed for pathological diagnosis for research purposes. The eyes were fixed in formalin, embedded in paraffin, and sectioned according to routine methods. All had at least 1 mm of optic nerve present. Individual sagittal sections containing the optic disk and contiguous nerve were incubated with naphthol AS-D chloroacetate, a substrate specific for the chloroacetate esterase staining method. A Poisson distribution was used to model an idealized randomly distributed mast cell population, and its fit measured with biometric techniques were employed to estimate the number of mast cells per unit volume. Briefly, the number of mast cells seen in a unit area of a slide was multiplied by the number of section thicknesses per unit depth, and then corrected for multiple countings by a factor dependent on the thickness of the sections and the average size of the mast cells (Figure 1). Student’s t test was used for comparisons between two groups and one-way analysis of variance for testing equality of multiple group means. A Poisson distribution was used to model an idealized randomly distributed mast cell population, and its fit measured with the Kolmogorov-Smirnov statistic.

RESULTS

Of the 66 eyes stained for chloroacetate esterase, 65 had one or more mast cells present in the optic nerve meninges or parenchyma. These were identified by the brilliant carmine staining of their cytoplasmic granules, the characteristic shape, and densely-packed evenly sized granules typical of connective tissue mast cells (Figure 2). In normal nerves from eyes studied at autopsy, mast cells were only found in the meninges. For the most part, mast cells were distributed in the dura of the nerves, and only rarely in the arachnoid. There was some indication of increased concentration

### TABLE 1. Optic Nerve Mast Cell Density

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Eyes</th>
<th>Mean Nerve Length (mm)</th>
<th>Mast Cells (per mm³)</th>
<th>Degranulation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>20</td>
<td>6.7</td>
<td>269.7 ± 64.1</td>
<td>4.7 ± 1.9</td>
</tr>
<tr>
<td>Anomalies*</td>
<td>7</td>
<td>3.6</td>
<td>15.2 ± 5.6</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Neoplasms†</td>
<td>12</td>
<td>5.6</td>
<td>341.8 ± 74.2</td>
<td>13.6 ± 5.2</td>
</tr>
<tr>
<td>Inflammatory‡</td>
<td>10</td>
<td>5.3</td>
<td>676.2 ± 210.6</td>
<td>4.8 ± 2.4</td>
</tr>
<tr>
<td>Phthisis§</td>
<td>4</td>
<td>7.2</td>
<td>392.1 ± 162.5</td>
<td>18.9 ± 14.3</td>
</tr>
<tr>
<td>Trauma¶</td>
<td>6</td>
<td>5.6</td>
<td>482.3 ± 98.6</td>
<td>44.7 ± 14.8</td>
</tr>
<tr>
<td>Vascular¶</td>
<td>7</td>
<td>4.9</td>
<td>595.8 ± 140.1</td>
<td>6.0 ± 3.9</td>
</tr>
</tbody>
</table>

Counts of mast cells within optic nerves from patients with various disorders, using the chloroacetate esterase staining method.

* Multiple congenital anomalies, Klippel-Trenaunay syndrome, multiple skeletal anomalies, trisomy 13, trisomy 18.
† Choroidal melanoma, retinoblastoma, carcinomatous meningitis of nerve, retinal pigment epithelium tumor.
‡ Cytomegalovirus, granulomatous uveitis, panuveitis, herpes zoster ophthalmicus, ocularcutaneous pempigoid and endophthalmitis, necrotizing scleritis, toxocara.
§ Endstage diabetic retinopathy, chronic retinal detachment, retinopathy of prematurity.
¶ Intraocular foreign body, ruptured globe.
¶ Branch retinal artery occlusion, branch retinal vein occlusion, central retinal vein occlusion, ischemic oculopathy.
Mast Cells in Human Optic Nerve

**Volume of meninges = volume of outer cylinder - volume of inner cylinder**

\[ V_{outer} - V_{inner} = \pi L (D/2)^2 - \pi L (d/2)^2 \]

**Correction factor (accounts for the fact that a single cell may be seen on more than one thin section)**

\[ \text{Cells per volume} = \frac{\text{Cells per area}}{\text{Thickness} \times \text{Thickness}} \]

**FIGURE 1.** Protocol for computing number of mast cells per unit volume from counts of slide sections. In this method, mast cells were modeled as spheres as assumed to be approximately 10 μm in diameter. The correction factor is needed to account for the fact that a single mast cell may occur on more than one slide, and thus be otherwise overcounted.

Of mast cells adjacent to meningeal vessels (Figure 3). When biometric techniques were used to estimate the total number of mast cells per unit volume of tissue, the density was as much as 2325 mast cells per mm³ with a mean of 269.7 ± 64.1 in normal nerves. There was a wide dispersion of mast cell counts between individual specimens. Although there was no specific correlation of mast cell number with specific disease etiology, there was a pattern seen, with normal nerves and nerves from fetuses aborted for congenital defects having fewer mast cells than those with inflammatory or vascular diseases (\( P = 0.026 \) and 0.024, respectively; Table 1). Eyes with trauma also had high numbers of mast cells, but this did not reach statistical significance. When the presence of inflammation on routine pathological examination was correlated with disease group, all eyes with recent trauma had some uveal inflammatory cells, which were predominantly nongranulomatous and in the choroid. Three eyes from patients with globe trauma, herpes zoster ophthalmicus, and endophthalmitis had low levels of optic nerve inflammation; in these cases, mast cells were not particularly co-localized with areas of inflammation. There was no association between mast cell number and the presence of optic atrophy or gliosis. Except for one eye with meningeal involvement from carcinomatous meningitis, there was no involvement of the optic nerve by any of the neoplasms studied. An eye from a patient with neovascular complications (rubeosis and neovascular glaucoma) of diabetic retinopathy also had a high concentration of mast cells (746.7/ mm³); neither this, nor any other eye, had neovascularization of the disc visible on histologic sections.

Mast cells were only rarely seen in the parenchyma of optic nerves (4 of the 66 patients [6.1%], Figure 4). These patients had evidence of prior eye disorders, specifically central retinal vein occlusion, branch retinal vein occlusion, neovascular glaucoma after trauma, and end-stage glaucoma after retinal detachment surgery. These parenchymal mast cells were often noted adjacent to vessels, particularly the central retinal artery and vein (Figure 5). Parenchymal mast cells were not seen in any optic nerves from fetuses, acutely traumatized globes, or donor eyes.

To estimate whether mast cells were randomly distributed, a histogram of distances between adjacent mast cells was constructed, measured in a longitudinal direction (Figure 6). The mean spacing between mast cells in the longitudinal direction was 289 μm, with a standard deviation of 334 μm. To assess whether a pattern of grouping was present, the data were compared with what would be expected if the mast cells were distributed randomly in the anteroposterior direction. Comparison between the observed distribution and a theoretical Poisson distribution was not significant (Kolmogorov-Smirnov statistic = 0.287).

Degranulated mast cells were frequently present, as identified with either stain by the presence of multiple extracellular granules (Figure 7). Although a chronically degranulated cell could not theoretically be detected, because of a lack of staining, the specimens with acute or partial mast cell degranulation were studied. There was a correlation between type of disease and percentage of degranulated mast cells, with eyes with a history of recent severe trauma having 44.7% ± 14.8% degranulated cells. Eyes with intraocular neoplasms and phthisical eyes had fewer degranulated mast cells (13.6% ± 5.2% and 18.9% ± 14.3%), whereas eyes with inflammatory and vascular disorders had about the same low level of degranulation as was seen in normal eyes or those from fetuses with congenital anomalies (4.7%). Analysis of variance showed the differences between groups to be highly significant (\( P < 0.001 \)).

Studies with acidified toluidine blue reproduced the staining pattern observed with chloroacetate esterase staining. Mast cells were readily identified by their
FIGURE 2. Mast cells in the meninges of human optic nerve from a patient dying of congestive heart failure. The polygonal cytoplasm, small rounded dark nucleus, and brilliant carmine granules are characteristic of mast cells using the chloroacetate esterase stain. (Original magnification × 315.)

metachromatic purple granules and slightly blue round nuclei (Figure 8). As with the chloroacetate esterase stain, mast cells were seen in the meninges of the optic nerve, often adjacent to blood vessels.

DISCUSSION

It has long been recognized that mast cells are distributed in certain ocular tissues. In the conjunctiva, they are partly responsible for atopic responses, such as allergic conjunctivitis or atopic keratoconjunctivitis. These conditions are typically treated with agents that block the physiological effect of mast cell granule contents, such as antihistamines, or with agents that block mast cell degranulation, such as cromolyn. Mast cells are also found in the conjunctiva, episclera, iris, ciliary body, and choroid, where there is evidence suggestive of a role in the pathogenesis of certain experimental

FIGURE 3. Mast cell adjacent to small vessel of the optic nerve meninges from a 66-year-old woman with chorioretinitis of unclear cause. The absence of a vessel near a mast cell may be a result of the section being parallel to and displaced from the axis of the vessel; this sectioning artifact may underestimate the number of perivascular mast cells (chloroacetate esterase; original magnification × 315).

block the physiological effect of mast cell granule contents, such as antihistamines, or with agents that block mast cell degranulation, such as cromolyn. Mast cells are also found in the conjunctiva, episclera, iris, ciliary body, and choroid, where there is evidence suggestive of a role in the pathogenesis of certain experimental

FIGURE 4. Parenchymal mast cells in an optic nerve from a 32-year-old man with neovascular glaucoma 15 years after trauma. There are no nearby inflammatory cells to indicate that the cell has entered the tissue from the intravascular space (chloroacetate esterase; original magnification × 50).

FIGURE 5. Mast cells in the parenchyma of an optic nerve from a 79-year-old woman with neovascular glaucoma after central retinal vein occlusion. The mast cells are easily seen adjacent to the central retinal vein (large arrow). Neutrophils (small arrow), which also stain for chloroacetate esterase, can be differentiated from mast cells by their distinctive cytoplasmic and nuclear morphology. In general, parenchymal mast cells are seen far less frequently than those in the meningeal coats of the optic nerve (chloroacetate esterase; original magnification × 200).
Mast Cells in Human Optic Nerve

Table 2 of Levene). In rat brain meningeal mast cells are distributed. In this study, their mean concentration is approximately 500 per mm³ (calculated from Figure 1). Mast Cells in Human Optic Nerve 3151 Studies, counts ranging from 10 to 60 cells per mm² of tissue section have been reported, which on a volume basis would be approximately 1000 to 6000 cells per mm³. Limitations of patient data collection precluded correlating mast cell number and certain phenotypic characteristics, particularly human leukocyte antigen groupings. Nevertheless, genetic variation of mast cell number may account for some of the patient-to-patient variability in mast cell number observed in these studies. Most of the variation is accounted for by the differences in the nature of the eye disorder. Presumptively normal nerves, such as those from fetuses or autopsy eyes, had the lowest number of mast cells (15.2 ± 5.6 and 269.7 ± 64.1 cells/mm³). In comparison, eyes from patients with inflammatory disorders, such as herpes zoster ophthalmicus, panuveitis, and Guillain-Barré syndrome, had the highest number of mast cells. Presumably cytokines, such as interleukins-3,4,5,6 and other interleukins released by inflammatory cells involved in the disease process in these eyes caused either migration or proliferation of mast cells, some of which ended up in the optic nerve meninges.

Mast cell degranulation results in the release of a variety of inflammatory mediators that may affect immunity and vascular permeability. In particular, cytokines released by mast cell degranulation, such as interleukins-3,4,5,6 and tumor necrosis factor-alpha may potentiate local inflammation, and histamine and other vasodilators may affect vessel tone and the state of the blood nerve barrier. In this study, degranulated mast cells (as a percentage of total mast cells) were present in highest numbers in eyes with recent trauma (44.7 ± 14.8%, compared with 4.7 ± 1.9% for normal optic nerves). This probably reflects a tendency for degranulation to occur as a result of mechanical factors. However, the histochemical techniques used are relatively insensitive to assessing degranulation, because cells that are devoid of granules are not stained. This would systematically underestimate degranulation, and make difficult the correlation of percentage mast cell degranulation with pathological processes resulting from release of mast cell mediators. Other techniques, such as immunohistochemical detection of surface immunoglobulin E receptors, may prove to be more sensitive in detecting degranulated mast cells.

Although in the current study most mast cells were located in the optic nerve meninges, they were also present in the parenchyma in certain pathological conditions. Mast cells are generally considered to be sessile, but they can be locally recruited in certain experimental and developmental situations. For example, we have shown that both the peripheral and central nervous systems of congenitally mast-cell–deficient strains of mice can be reconstituted with mast cells by bone marrow transplant. In the current study,
patients with vascular disorders, especially central retinal vein occlusion and branch retinal vein occlusion, had a particularly high concentration of mast cells (595.8 ± 140.1/mm³), which was almost as high as those with inflammatory conditions (676.2 ± 210.6/mm³). In three patients with neovascular complications of vessel occlusion and trauma, and one with end-stage glaucoma, mast cells were found in the parenchyma. Mast cells are closely associated with blood vessels, so it is not surprising that neovascularization associated with those or other disorders may also recruit mast cells. It has been shown that mast cells migrate during normal development from pia mater along blood vessels to perivascular locations within the thalamus. Alternatively, nerve growth factor, which increases numbers of mast cells, may be responsible for locating foci of mast cells near vessels. It is possible that the pathological conditions occurring in vascular occlusive or other diseases may result in a recapitulation of these ontogenetic processes.

The role of mast cells in the optic nerve is unclear. Although they may play a role in regulating vascular permeability, diameter, and immunity, there is little evidence for this within the optic nerve, nor the rest of the central nervous system. Mast cells in and around the optic nerve may have different functional implications from those found elsewhere in the central nervous system. In the brain, the surface to volume ratio of meninges to parenchyma is clearly much lower, and the average distance of cerebral tissue from meningeal surface is much greater than in the optic nerve, where the distance between an optic nerve axon or glial cell and the dura or arachnoid is never more than approximately 1.5 to 2 mm (half of the myelinated optic nerve diameter of 3 to 4 mm). Therefore, the physiological and pathological significance of mast cells in the optic nerve is likely to be greater than in the brain. For example, localized tissue edema from mast cell degranulation may affect the more peripheral optic nerve parenchyma, possibly interfering with axonal action potential conduction. Mast cells that are aberrantly within the nerve parenchyma may also affect nerve conduction, either by the same mechanism, or through the direct action of granule contents on nearby astrocytes. The reason for the presence of mast cells in this location is unclear; we have shown that mast cells survive on cultured astrocyte monolayers, which suggests that optic nerve astrocytes may support mast cell survival there. Finally, evidence from work with experimental demyelinating disorders suggests that mast cells may play a role in autoimmunity within the nervous system. It is possible that mast cells located in the meninges of the optic nerve, and within the optic nerve parenchyma itself in patients with certain ocular disorders, may be pathogenically significant in certain optic nerve allergic and inflammatory conditions, and could be investigated by manipulating mast cell activation in experimental models of optic neuritis.

Key Words
mast cell, optic nerve, meninges, chloroacetate esterase, optic neuritis
Mast Cells in Human Optic Nerve

References


