ORIGINAL ARTICLES – Laboratory science

Distribution and characterisation of rat choroidal mast cells

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Abstract

Despite the implication that choroidal mast cells are involved in the onset of experimental autoimmune uveoretinitis (EAU), a widely used animal model of uveoretinitis, little is known of these cells. In the present study the distribution, total number, regional density, and phenotype of choroidal mast cells were examined in Lewis, Wistar Furth, PVG/c, and brown Norway rats. Choroidal mast cells were predominantly associated with arteries and arterioles of more than 30 μm diameter which lie in the outer (scleral) choroid. The density of mast cells was greatest in the posterior choroid with density diminishing anteriorly. The choroid of male Lewis rats contained significantly greater number of mast cells than that of females ($p < 0.01$). Histochemical (Alcian blue/safranin) and immunohistochemical (anti-rat mast cell protease I and II monoclonal antibodies) studies revealed choroidal mast cells were of the connective tissue type. However, granule proteinase content appeared less than that of well characterised connective tissue mast cell populations such as those in mesentery and skin. Lewis rats exhibited the highest density of choroidal mast cells (23.6 (SD 1.2)/ mm^2), Wistar Furth approximately half that of Lewis (13.5 (0.7)/ mm^2) while PVG/c and brown Norway rats had very low densities (3.06 (0.3); 1.95 (0.2)/ mm^2 respectively). These studies provide valuable choroidal mast cell data for rats which may have implications for our understanding of experimental models of intraocular inflammation and clinical uveitis.

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Mast cells are central to IgE mediated immediate hypersensitivity reactions and have been increasingly implicated in a wide range of inflammatory responses.^{1,2} Mast cells may also participate in a variety of normal physiological processes.³

The presence of mast cells in the lid, conjunctiva, and uveal tract of the mammalian eye has been reported for a number of species including rats,^{4–6} guinea pigs, rabbits, monkeys,⁴ and dogs.⁷ Mast cells within human ocular tissue have not been widely studied; however, they are present in variable numbers throughout the uveal tract – namely, the iris,⁸ ciliary body,⁹ and choroid.¹⁰

Allansmith and associates⁶ estimated the number of mast cells within various tissue compartments of the rat eye and adnexa by analysis of

tissue sections. These authors reported the vast majority of mast cells were located in the external structures – the eyelids, conjunctiva, and orbit. Mast cells observed within the uveal tract were largely restricted to the choroid.⁶ While early investigations primarily studied the presence and variation in the number of choroidal mast cells between species, some information pertaining to the distribution of mast cells within the choroid has been reported. Smelser and Silver,⁵ using a novel flat mount technique, reported an antero-posterior gradient of choroidal mast cell density in a number of species. Godfrey⁹ described rat choroidal mast cells in a periarterial location. While the distribution of mast cells within extraocular tissues is consistent with the usual strategic location below surfaces exposed to the external environment¹¹ and their role in IgE mediated responses, the function of mast cells within the uveal tract is still unclear.

Distinct mast cell subpopulations are distinguishable by functional and morphological characteristics.^{12,13} In the rat, two phenotypes are widely recognised: the connective tissue mast cell (CTMC), found predominantly in the skin, peritoneal cavity, and other areas of connective tissue¹⁴ and the mucosal mast cell (MMC) which occurs in the mucosa of the gastrointestinal^{15,16} and respiratory tracts.^{17,18} These phenotypes may be partially distinguished by the histochemical staining characteristics of their proteoglycan content,¹⁶ but more definitively by analysis of serine proteinases within the intracellular storage granules.¹⁹

The phenotype of choroidal mast cells has not been established to date, although it may be important to the role of these cells in intraocular immunological processes and inflammation due to, for example, the differing mediator production²⁰ and T cell dependence¹⁴ of the two subpopulations.

Variations in choroidal mast cell numbers during the course of experimental autoimmune uveoretinitis (EAU) in Lewis rats led de Kozak and associates²¹ to propose that choroidal mast cells have a role in the onset of this inflammatory disease. Supportive evidence of such a role was provided by Mochizuki and colleagues²² who demonstrated a positive correlation between choroidal mast cell numbers and EAU susceptibility in a number of rat strains. Highly susceptible strains, such as Lewis, had up to a fivefold greater density of choroidal mast cells than strains of low susceptibility.

As a prerequisite to investigating the function

of choroidal mast cells in intraocular inflammatory disease it was considered appropriate to obtain more detailed information on the normal distribution, location, and phenotype of these cells in a number of rat strains.

Materials and methods

Male and female Lewis and female PVG/c, brown Norway, and Wistar Furth rat strains (specific pathogen free), aged 10–12 weeks, were obtained from the Animal Resources Centre, Murdoch, Western Australia. Six animals of each strain were used to obtain choroidal mast cell density data from flat mounts. For all strains, other than Wistar Furth, both eyes were examined and an animal mean determined. For Wistar Furth, one eye from each of six animals was used to generate choroidal mast cell density data. Three animals each of Lewis, Wistar Furth, PVG/c, and brown Norway strains were used to determine the histochemical and immunohistochemical (albino strains only) staining characteristics of choroidal mast cells in paraffin sections. A further group of Lewis rats was used for immunohistochemical staining of tissue whole mounts (choroid and mesentery).

Following sodium pentobarbitone induced anaesthesia, animals were perfused with cold heparinised phosphate buffered saline, followed by cold 4% paraformaldehyde in sodium cacodylate buffer. Eyes were rapidly enucleated and further fixed for 6–8 hours, then transferred to 70% ethanol for 12–18 hours. Eyes were prepared either as choroidal flat mounts or embedded in paraffin wax. Mesentery spread mounts were prepared from portions of ileum and processed in a similar manner.

HISTOCHEMICAL STAINING OF CHOROIDAL FLAT MOUNTS

Rat mast cell phenotypes can be partially distinguished by differential staining of granule proteoglycans following staining with a sequence of Alcian blue followed by safranin. The granules of mature CTMC have a high heparin content and stain red due to safranin displacing Alcian blue from heparin and binding in its place. The more highly sulphated proteoglycans of MMC and immature CTMC stain blue as Alcian blue is not displaced from these proteoglycans by safranin.

Flat mount preparations, consisting of the choroid and attached sclera, were rehydrated in distilled water for 5 minutes and then processed as a single free floating unit. Alcian blue/safranin staining was performed as previously described.²³ Briefly, free-floating preparations were acidified in 0.7 M HCl (5 minutes) followed by immersion in 0.1% Alcian blue (Gurr, England) in 0.7 M HCl (60 minutes). Preparations were then briefly rinsed in distilled water and immersed in 0.5% safranin (Hopkins and Williams, England) in 0.125 M HCl (5 minutes). Finally, preparations were dehydrated through graded alcohols, cleared in toluene, and mounted. Mesentery spread mounts were also stained using the protocol applied to choroidal flat mounts.

Owing to pigmentation in PVG/c and brown Norway strains, bleaching of choroidal prepara-

tions was necessary to enable visualisation of mast cells. Before Alcian blue/safranin staining choroid preparations were bleached with 5% chromic acid (3×25 minutes), rinsed in distilled water (5 minutes) and further bleached in 5% oxalic acid (10 minutes). The preparations were then rinsed in distilled water (5 minutes). Mast cell numbers in Alcian blue/safranin stained preparations were compared in bleached and unbleached choroidal flat mounts from six albino animals (Wistar Furth) to determine the effects of the bleaching protocol. No significant difference was found ($p > 0.53$).

Toluidine blue staining was performed on choroids from a further two Lewis rats. Preparations were placed in 0.5 M HCl (5 minutes) before staining in 0.5% toluidine blue (Hopkins and Williams, England) in 0.5 M HCl (10 minutes). Preparations were then rinsed (3×distilled water), dehydrated, and mounted.

HISTOCHEMICAL STAINING OF PARAFFIN SECTIONS

A number of eyes were embedded in paraffin wax using routine methods. Sections (5 μ m) were cut and mounted on slides. Following dewaxing and rehydration sections were stained with Alcian blue/safranin and toluidine blue following the protocols used for choroidal flat mounts.

IMMUNOHISTOCHEMICAL CHARACTERISATION OF CHOROIDAL MAST CELLS

Although histochemical staining differentiates mature CTMC from MMC and immature CTMC a more definitive method to distinguish mast cell phenotype involves analysis of the distinct serine proteinases within cytoplasmic granules. Rat mast cell protease I (RMCP I) which occurs in CTMC and rat mast cell protease II (RMCP II) present in MMC can be detected by immunohistochemical staining.

Paraffin sections were stained with anti-RMCP I and anti-RMCP II according to a method

Figure 1 Distribution of mast cells in the Lewis rat choroid with major arterial vessels shown. This figure demonstrates the distribution of mast cells within a single choroid, one dot representing a single mast cell. This distribution is representative of all choroids examined. Note the superior and inferior long posterior ciliary arteries and large branch supplying a major portion of the nasal regions. Note also, the increased density of mast cells within the nasal regions.

Figure 2 Photomicrograph of a flat mount of female Lewis choroid (toluidine blue). Note the abundance of mast cells associated with arterial vessels (A) and the absence of mast cells associated with venous vessels (V); $\times 45$.

previously described.²¹ The extraocular muscles and surrounding connective tissue of the rat contain large numbers of mast cells, which served as an internal positive control for RMCP I staining. Sections of small intestine prepared from rats infected with *Nippostrongylus brasiliensis*, which display a characteristic MMC hyperplasia,²⁴ provided positive control sections for RMCP II staining. One section of this material was included on each slide prepared for RMCP II immunostaining. Negative controls were prepared by substituting phosphate buffered saline in place of primary monoclonal antibodies. A number of sections were examined and then counterstained with Alcian blue according to the first stage of the Alcian blue/safranin sequence. In addition, to determine the proportion of choroidal mast cells expressing RMCP I, choroidal flat mounts were prepared, immunohistochemically stained for RMCP I and counterstained with Alcian blue.

MAST CELL DENSITY AND DATA ANALYSIS

Mast cell numbers were obtained by counting stained cells in entire choroidal flat mounts. Areas of the choroidal flat mounts were determined using a digitiser pad in conjunction with an area calculating software package (Sigma-

Figure 3 Posteroanterior gradient of mast cell density in the choroid of the Lewis rat. Columns represent mean with SEM.

Scan; Jandel Scientific, USA). Statistical significance was tested using Student's *t* test.

Results

DISTRIBUTION, LOCATION, AND DENSITY OF CHOROIDAL MAST CELLS

The choroidal vascular supply was consistent within and between strains of rat. A single large branch of the ophthalmic artery pierced the sclera nasal to the optic nerve head and bifurcated, forming superior and inferior long posterior ciliary arteries (LPCA) (Fig 1). The LPCA run anteriorly to join the major arterial circle of the iris. Posteriorly, the LPCA were located within a depression on the internal aspect of the sclera, while anteriorly the LPCA were situated within the dense connective tissue of the sclera. Normally one, and occasionally a second, major branch arose from either LPCA very close to the optic nerve head and supplied a large portion of both the superior and inferior nasal quadrants of the choroid (Fig 1). Small arterial branches of the LPCA supplied the remainder of the choroid.

In Lewis rats mast cells were irregularly distributed throughout the choroid (Fig 1). Large numbers of mast cells were present in the connective tissue surrounding the branch of the ophthalmic artery, at the point where it penetrates the sclera, and the two LPCA for a short distance after bifurcation. Consistently larger numbers of mast cells were observed along the inferior LPCA. In addition, the inferior LPCA had a region of increased mast cell density near its iridial termination (approximately 50 mast cells along the final 1 mm). Mast cell density was consistently greater in the nasal portion associated with the major arterial branch(s) supplying this region. In addition, whole mounts of Lewis anterior uveal tract were examined; these revealed low numbers of mast cells present in the ciliary body (approximately 30 per eye) and iris (0–1 per eye).

On high power examination of stained choroidal flat mounts, mast cells were observed predominantly in a perimural location associated with arteries and arterioles greater than 30 μm in diameter (Fig 2). In particular they were arranged along the lateral and not the sclerad or vitread margins of vessels. This location was confirmed in histological sections.

The density of mast cells within the choroid of Lewis rats varied in an anteroposterior manner (Fig 3), density being greatest (42/mm²) in the equatorial, or middle, regions of the choroid. From this region, density diminished rapidly to a minimum (12/mm²) at the anterior margin of the choroid. A distinctive focal region of high mast cell density (40/mm²) occurred around the optic nerve head.

In paraffin sections, mast cells were observed in association with the large and small arteries of the choroid and were largely absent from the choriocapillaris (Fig 4). This contrasts with the location in mesentery spread mounts where mast cells were distributed evenly throughout the tissue with small numbers associated with veins and venules larger than 25 μm (Fig 5a, b).

Sexual dimorphism was evident in the Lewis

Figure 4 Safranin positive mast cell adjacent to a choroidal arteriole in the Lewis rat; R=retina, C=choroid (paraffin embedded, Alcian blue/safranin); $\times 1100$.

Figure 5a Safranin positive connective tissue mast cells in a mesentery spread mount (Lewis rat). Note the pattern of distribution of mast cells both close to and distant from a large venule (Alcian blue/safranin); $\times 100$.

Figure 5b RMCP I positive mast cells in mesentery spread mount; $\times 250$.

Figure 6 Choroidal flat mount (Lewis rat) demonstrating periarteriolar mast cells containing a mixture of blue and red staining granules (Alcian blue/safranin); $\times 350$.

Figure 7 Mast cells in a flat mount preparation (Lewis rat). Note the predominantly periarteriolar location (toluidine blue); $\times 250$.

Figure 8a Blue staining mucosal mast cells in the small intestine of *Nippostrongylus brasiliensis* infected rat following Alcian blue/safranin sequence; $\times 100$.

Figure 8b Mucosal mast cells in the small intestine of *Nippostrongylus brasiliensis* infected rat demonstrating positive RMCP II immunostaining; $\times 100$.

Figure 9a RMCP I positive mast cells adjacent to arterioles in the choroid (Lewis rat, paraffin embedded); $\times 1100$.

Figure 9b Positive control section (paraffin embedded mesentery) immunostained for RMCP I. Note the intensity and uniformity of immunopositive granules throughout the cytoplasm (Lewis rat); $\times 400$.

Figure 10 Choroid flat mount (Lewis rat) sequentially stained with anti-RMCP I (brown) and Alcian blue (blue/green). Note the variable quantity of RMCP I positive granules throughout the cytoplasm; $\times 250$. Inset; higher magnification; $\times 600$.

Figure 11 Choroid flat mount (brown Norway rat) demonstrating blue staining mast cells (arrowheads); $\times 200$.

rats where both total choroidal mast cell number and density (Table 1) were both significantly greater in males ($p < 0.01$ in both cases). No observable difference existed in the distribution and microanatomical arrangement of mast cells throughout the choroid between sexes.

MAST CELL MORPHOLOGY, HISTOCHEMISTRY, AND PHENOTYPE

Choroidal mast cell profiles were clearly evident in flat mount preparations and were most commonly ovoid ($7 \times 20 \mu\text{m}$), although circular ($12\text{--}15 \mu\text{m}$ diameter) and elongated ($5 \times 30 \mu\text{m}$) profiles were occasionally noted (Figs 6 and 7). In general, the shape of choroidal mast cells appeared related to two factors, the Alcian blue/safranin staining characteristics and the proximity to arterial vessels. Periarterially located mast cells containing predominantly blue staining granules were most frequently thin and elongated. However, mast cell profiles became increasingly rounded as the distance from vessels became greater or the degree of safranin staining increased (Figs 6 and 7). A minor subpopulation of small, round ($7\text{--}10 \mu\text{m}$ diameter), blue staining mast cells was observed distant to arterial vessels, most commonly in the anterior portions of the choroid.

The majority of mast cells (89%) contained a variable mixture of both red and blue stained granules ('mixed staining') (Fig 6), blue staining cells constituted approximately 6% of choroidal mast cells, while red staining cells made up approximately 5% of the total. Choroidal mast cell granules in paraffin sections were predominantly safranin positive (Fig 4), only rarely

were blue staining mast cells observed, as expected from their rarity in flat mounts.

Mast cells in toluidine blue stained material exhibited intense staining and distinctive purple metachromasia (Fig 7). Details of intracellular granules and nuclear profiles were often obscured due to the intensity of staining (Fig 7), although occasionally, a large pale central nucleus was observed. The margins of mast cells within toluidine blue stained material were smooth and clearly delineated. Evidence of mast cell degranulation was minimal, only 3.6% (SEM 0.25%) of mast cells displayed a degranulated appearance in toluidine blue stained paraffin sections.

During the course of this study the question arose whether both staining protocols were visualising all mast cells present in rat choroid. Therefore, a comparison of the ability of the Alcian blue/safranin and toluidine blue staining protocols to determine choroidal mast cell number was performed. No significant difference was observed between the total number of choroidal mast cells visualised by toluidine blue and Alcian blue/safranin in consecutive serial sections of Lewis choroid.

Staining with anti-RMCP II antibodies failed to reveal any immunopositive cells within the choroid or extraocular tissues with the exception of some limbal conjunctival mast cells. Control tissue (*N. brasiliensis* infected gut) displayed positive staining of MMC (Fig 8a, b).

Immunohistochemical studies of histological sections and flat mounts both revealed choroidal mast cells to be RMCP I positive and generally associated with large and small arterial vessels (Fig 9a). In addition, RMCP I positive mast cells were observed surrounding limbal vessels and in extraocular tissue. The immunostaining intensity of choroidal mast cells was variable and often less intense than that displayed by mast cells in positive control tissue such as lymph node or mesentery (Fig 9b). Choroidal flat mounts immunostained for RMCP I and counterstained with Alcian blue provided a novel method of integrating histochemical and immunohistochemical procedures. In these preparations all mast cells contained at least a small number of RMCP I positive granules; however, a wide range in the content of RMCP I positive granules (brown reaction product) was present (Fig 10). In addition, peripherally located RMCP I negative granules which were Alcian blue positive (Fig 10) corresponded to the peripheral blue granules seen following Alcian blue/safranin staining (Fig 6). Mast cells within the choroid contrasted with those in mesentery spread mounts which had a uniformly high content of RMCP I positive granules (Fig 5b) which as indicated above contained almost exclusively safranin positive granules (Fig 5a).

STRAIN VARIATIONS IN CHOROIDAL MAST CELL NUMBER

Previous studies have suggested strain specific variations in choroidal mast cell number may be implicated in the susceptibility to EAU, therefore we chose to re-examine this issue with regard to mast cell heterogeneity in low, medium, and

Table 1 Mean mast cell number and densities in the choroid and mesentery of the rat strains examined

Strain	Mean choroidal mast cell number (SEM)	Mean mast cell density, cells/mm ² (SEM)	
		Choroid†	Mesentery‡
Lewis (male)*	1813 (116)	37.0 (3.3)	ND
Lewis (female)*	1180 (51)	23.6 (1.2)	102 (7)
Wistar Furth	610 (52)	13.5 (0.7)	ND
Brown Norway	106 (9)	3.06 (0.3)	110 (7)
PVG	69 (8)	1.95 (0.2)	97 (8)

*Significant difference between male and female ($p < 0.01$).

†Significant differences exist between all strains ($p < 0.001$, except PVG/c and BN, $p < 0.01$).

‡No significant difference between any strains; ND = not determined.

high IgE responsive rat strains: Lewis (low), PVG/c and Wistar Furth (moderate), and brown Norway (high). The choroid of Lewis rats contained the greatest number of mast cells; however, in comparison the choroid of both PVG/c and brown Norway rats contained low numbers of mast cells (Table 1). The number of choroidal mast cells in Wistar Furth rats was approximately half that of Lewis rats. These strain variations in mast cell density are apparently tissue specific as no significant difference was observed between the density of mast cells within the mesentery spread mount preparations from Lewis, PVG/c, or brown Norway rats (Table 1).

Choroidal mast cells in all strains demonstrated the same periarterial location described previously for Lewis rats. Mast cells within the choroid of Wistar Furth rats displayed the same general distribution seen in Lewis rats; however, in those strains of rat with a very low number of choroidal mast cells (PVG/c, brown Norway) the distribution was most commonly restricted to the large arterial vessels in the region of the optic nerve head. In brown Norway rats a small number of mast cells¹⁵⁻²⁰ were observed in the mid-choroidal region of the nasal quadrants, associated with the large branch of the long posterior ciliary arteries supplying this area. In PVG/c rats choroidal mast cells were restricted almost exclusively to the proximal portions of the large arterial vessels and the long posterior ciliary arteries.

Mast cells in bleached choroidal flat mounts failed to display safranin positive granules (Fig 11); however, in paraffin sections of PVG/c and brown Norway rat eyes choroidal mast cells displayed characteristic safranin positive as described in sections of Lewis choroid (not shown).

Discussion

The present study was prompted by the increasing awareness of the diverse role of mast cells beyond classic IgE mediated hypersensitivity, together with the need for a more precise knowledge of their distribution, location, and phenotype in the choroid. The present study expands previous reports by providing, not only a more detailed account of the strain specific variations in choroidal mast cell density, distribution, and microanatomical location in rats but, moreover, presents novel data with regard to the histochemical and immunohistochemical phenotype of these cells.

The physiological significance of the association of mast cells with arterial vessels and the regional variation in distribution is unclear at this stage. It is possible, however, that local regulation of blood flow within the choroid, in response to the requirements of the overlying retina, may be among the normal physiological roles of these cells. Interestingly, CTMC have been identified along the margins of the middle meningeal artery in the rat dura mater,²⁵ a tissue analogous to the sclera. It has been suggested that mast cells in this region may have a role in the regulation of blood flow and the pathogenesis of headache,^{26,27} although this is a matter of controversy. Local control of blood flow and secretory activity by mast cells has been demonstrated in the rat adrenal gland²⁸ and both rat²⁹ and hamster ovary.³⁰

Degranulation of periarterial mast cells in the hamster cheek pouch results in diapedesis of leucocytes exclusively from 'downstream' post-capillary venules. Leucocytes then migrate toward adjacent arterioles along chemotactic gradients generated by the mast cell degranulation.³¹ The results of the present study are consistent with speculation that the activation of choroidal mast cells reported at the onset of EAU²¹ may result in mediators being carried downstream from the long posterior ciliary arteries to the extensive capillary beds of the ciliary body,³² where extravasation of inflammatory cells may be effected. It is possible a mechanism such as this may mediate the large scale inflammatory cell infiltration of the iris, ciliary body, and anterior chamber observed in EAU.³³ As both the iris and ciliary body of Lewis rats contain few mast cells, local release of mast cell mediators seems an unlikely explanation of such a response. It is interesting to note that rabbits, which have little connection between the choroidal circulation and that of the anterior segment, develop only minimal inflammatory infiltration of the anterior chamber during EAU.³⁴ Activation of mast cells has been shown to result in the production of a range of cytokines,^{35,36} which if released from choroidal mast cells, may have a 'downstream' effect on the extensive network of macrophages and major histocompatibility class II antigen bearing dendritic cells in the anterior uveal tract.^{37,38}

It is interesting to note the presence of mast cells in the vascular and connective tissue surrounding both the eye and brain. Mast cells have also been identified within selected regions of brain parenchyma,³⁹ whereas mast cells are absent from the neural retina. Furthermore, the retina is isolated from the mast cell rich choroid by the retinal pigment epithelium. These factors may indicate the need to protect the retinal vasculature and delicate photoreceptors from the action of mast cell mediators.

Sexual dimorphism in choroidal mast cell number has not previously been described. However, sexual dimorphism of mast cell content has been reported for a number of tissues, including rat peritoneal cavity,^{40,41} hamster and mouse harderian gland,^{42,43} and rat brain.³⁹ These tissues, however, differ from the choroid as mast cell content is invariably higher in females than in males. The reduced number of mast cells in these

tissues has been attributed to an androgen dependent mechanism.⁴²⁻⁴⁴ The functional significance of higher choroidal mast cell numbers in males is uncertain but may affect the susceptibility to, or the severity of, intraocular inflammation.

The presence of safranin positive granules within choroidal mast cells is strongly suggestive of CTMC phenotype. However, the presence of RMCP I positive cytoplasmic granules and absence of RMCP II immunostaining is more definitive evidence of the CTMC phenotype of choroidal mast cells. The coincidence of intense staining for RMCP I and the exclusively safranin positive nature of mesenteric mast cells suggests RMCP I may be restricted to safranin positive cytoplasmic granules. It is possible the Alcian blue positive/safranin negative and Alcian blue positive/RMCP I negative granules represent immature granules which have not yet attained heparin and RMCP I contents.⁴⁵⁻⁴⁶ The variable and thus, perhaps, reduced content of RMCP I within choroidal mast cells could serve to limit bystander damage, resulting from the proteolytic activity of this mediator,⁴⁷⁻⁴⁸ to the delicate cells in the immediate microenvironment such as the retinal pigment epithelium, an important component of the blood-ocular barrier. The presence of heparin and the susceptibility to compound 48/80 reported for rat choroidal mast cells by Godfrey⁹ are both features suggestive of CTMC phenotype and concur with the findings of the present study.

In Lewis rats a minor subpopulation of unusually small mast cells which contain exclusively Alcian blue positive granules was located distant to arterial vessels. The features of these mast cells suggest they were in an early stage of maturation from mast cell precursors.⁴⁵⁻⁴⁶ Although the lifespan of rat CTMC is greater than 6 months,⁴⁹ the presence of these small blue staining cells may indicate that mast cell precursors are being continually recruited, possibly from venous vessels,⁵⁰⁻⁵¹ in the anterior choroid, from where they may subsequently migrate to their periaarteriolar location and assume a more characteristic mature form.

The rat strains chosen in the present study demonstrate a range of IgE responsiveness and susceptibility to EAU. For example, Lewis rats are highly susceptible, PVG and Wistar Furth moderately susceptible, and brown Norway exhibit low susceptibility to EAU,⁵²⁻⁵³ while IgE production in response to antigenic stimulation shows an inverse trend.⁵⁴⁻⁵⁷ In the present study these strains were examined to determine the possibility of an association between IgE responsiveness, choroidal mast cell number, and EAU susceptibility. The mean total number of mast cells within the choroid of Lewis rats determined in the present study (1180 (SD 51)) is similar to that reported by Godfrey.⁹ A discrepancy exists, however, between the choroidal mast cell density determined in the present study (23.6 (1.2)/mm²) for Lewis rats and the value reported (50.2 (5.4)/mm²) by Mochizuki and associates,²² a value which is close to the mast cell density in the posterior choroid, possibly indicating incomplete sampling of the choroid. A possible explanation for the previously reported density in brown Norway (0.2 (0.1)/mm²)²² being lower

than our value (3.06 (0.3)/mm²) may be the bleaching protocol employed by Mochizuki and co-workers²² which, in our hands, caused extensive disruption of choroidal mast cell morphology. Moreover, the fixation protocol employed (10% formalin) is known to reduce the number of stained mast cells.⁵⁸ Choroidal mast cell numbers or density in PVG/c and Wistar Furth have not been reported before the present study.

The results of the present study are in agreement with the results of Mochizuki and colleagues²² which suggest that strain specific variation in the choroidal mast cell density is tissue specific. However, the results of this study are not consistent with the suggestion that susceptibility to EAU is directly correlated with choroidal mast cell number.²² The moderate responsiveness of PVG/c rats does not reflect the choroidal mast cell number of this strain which was the lowest of the strains examined in the present study. Indeed, the susceptibility to EAU shows an inverse relation to IgE responsiveness. Studies performed in this laboratory to investigate the role of choroidal mast cell dynamics during EAU in rat strains of differing susceptibility indicate their importance in the pathogenesis of EAU may be strain specific.⁵⁹

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