The effect of endotoxin-induced intraocular inflammation on the rat lens epithelium

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Intraocular inflammation induced by an intravitreal injection of Shigella endotoxin into the rat eye produces early changes in the number of dividing cells of the lens epithelium and affects the organization of the meridional rows. A depression in mitotic activity in the germinative zone is observed during the first 24 hr after injection. At 48 hr, despite the continued mitotic inhibition in the germinative zone, an increase in cell division occurs in the central zone. By 72 hr, the germinative zone mitosis reappears and exceeds control values, whereas the central zone mitotic activity returns to normal. At that time mitotic figures are found in the transitional zone. Disorganization of the meridional rows is seen as early as 12 hr after injection (the first time period observed) and reaches a peak by 48 hr. During the next 5 days, however, the severity of the disorganization diminishes. By the seventh day the rows appear, for the most part, fully recovered, and the mitotic activity reaches normal or near-normal levels in all regions. The details of these observations and their possible relationship to inflammatory cataracta complicata are discussed.

Key words: intraocular inflammation, lens epithelium, mitosis, meridional rows, cataracta complicata, rat, endotoxin, migration

Intraocular inflammation from varying etiologies has been reported to exert dramatic cytological effects on the lens epithelium. Classically these include hyperplasia of the epithelial population and migration of the cells posteriorly.1–6 Unfortunately, the experimental studies of the effects of inflammation on the lens generally involved chronic uveits or observations made long after the causative agent was introduced. The present study was undertaken to determine the early effects of an intraocular inflammation caused by a relatively short-acting.7–8 inflammatory agent, bacterial endotoxin, on the lens epithelial cell population.

Materials and methods

Shigella flexneri endotoxin (Difco laboratories, Detroit, Mich.) (0.2 μg in 1.0 μl of Earle’s balanced salt solution) was injected intravitreally into one eye of 10-week-old male Columbia-Sherman rats according to the procedure described previously.8 The contralateral eye received only the vehicle. The rats were then examined by slit-lamp biomicroscopy at intervals during the first day and daily thereafter. Following sacrifice, at times indicated by protocol, the eyes were enucleated and fixed in Carnoy’s solution (glacial acetic acid: absolute ethanol, 1:3) for at least 24 hr followed by an equal length of time in 70% ethanol. Then either the eyes were embedded in paraffin and sectioned at 4 μm or whole mounts of the lens epithelium were prepared according to the method of Howard9 as modified by Rothstein.10

The total number of mitotic figures per whole mount preparation was determined for cells occupying the central, germinative, and transitional zones of the epithelium. The germinative zone

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Fig. 1. Photomicrographs illustrating the semiquantitative manner in which meridional row disorganization was assessed. A number ranging from 0 to 4, depending on the degree of misalignment of the rows, was assigned to each preparation. In those preparations displaying variable disorganization, the most and the least disorganized areas were scored and the values averaged. (×250.)

was considered to include the cells occupying a circumferential band, the outer border of which began 10 cell diameters from the meridional rows (transitional zone) and the inner margin of which was demarcated by cells 70 cell diameters from the meridional rows. The inner area bordered by the germinative zone constituted the central zone. All the mitotic cells in each region were counted. Partial preparations or those with obvious cell loss were discarded.

In experiments designed to monitor possible cellular migration, tritiated thymidine (3H-Tdr) autoradiography was used. The labeled precursor was injected intraperitoneally (1.0 μCi/gm body weight; 3.0 Ci/mM) immediately following the intravitreal injections of endotoxin and vehicle. The animals were then sacrificed at 4, 24 and 48 hr after injection. The resulting whole-mount preparations were coated with NTB-2 autoradiographic emulsion. After 5 weeks of exposure the autoradiograms were developed (Microdol-X), and the whole mounts were stained with hematoxylin.

The relative organization of the meridional rows was determined by scoring the degree of misalignment of the rows in a semiquantitative manner (Fig. 1). Since the meridional rows in one area of the preparation would often differ in the degree of disorganization from those in another, the most disorganized and the least disorganized regions were scored and the values averaged. The average value thus obtained represented the degree of disorganization found in the entire lens epithelium.

Results

Fig. 2 shows the effect of inflammation on the mitotic activity in the central zone (2, A) and the germinative zone (2, B) of epithelia taken from lenses at various times following the intravitreal injection of *Shigella* endotoxin. Fig. 2, A, clearly shows that a stimulation of cell division in the central zone (an area usually devoid of mitosis) occurred in most preparations 24 hr following endotoxin administration. Reaching a peak at 48 hr, the mitotic activity was greatly reduced by 72 hr and returned to control levels by 6 days. The germinative zone (Fig. 2, B) normally had a great deal of proliferative activity but as a
result of the inflammation, there was a definite reduction in the number of dividing cells (Fig. 3). This inhibition of cell division was first observed at 12 hr and lasted at least 48 hr. Seventy-two hours after the endotoxin had been introduced, the mean number of mitotic figures in the lenses of endotoxin-treated eyes was twice that of control values. This was followed at 120 hr by a second but generally less severe depression in mitotic activity and eventual recovery by 14 days. Concomitant with the increase in mitosis in the germinative zone, mitotic cells would frequently appear in the transitional zone (the usually mitotically quiescent cell population adjacent to the meridional rows) (Fig. 4). The time course of this proliferative response is shown in Fig. 5.

To eliminate the possibility that some of the proliferative changes in the various zones during inflammation might be due to migration of cells with a mitotic potential from the germinative zone, H-Tdr autoradiography was used (see Methods). It was observed that the labeling index of treated and control eyes in the central zone was similar at 24 and 48 hr after injection, indicating that at those times migration of the labeled cell population from the germinative zone into the central zone had not occurred. Labeled cells were also absent from the meridional rows even at 48 hr.

The effect of inflammation on the relative alignment of the meridional rows was also examined with the same preparations which generated the data shown in Figs. 2 and 5. A disorganization of the meridional rows appeared to begin at about 12 hr, reaching a peak by 48 hr (Fig. 6). Interestingly, the study indicates that the meridional rows reconstitute, for the most part, returning to normal by 7 days. Fig. 7, A and B, shows the disorganization of the meridional rows as seen in cross-section or on a whole-mount preparation 48 hr after endotoxin treatment. Five days later the meridional rows appeared normal from both perspectives (Fig. 7, C and D).

The 0.2 µg dose of endotoxin used in this study failed to produce clinically detectable cataractous changes in the eyes of six animals which were examined periodically by slit-lamp biomicroscopy for 6 months.

Discussion

Although the precise effect of inflammation on the cell cycle remains to be determined, there is no question that the change in mitotic activity must be due, at least in part, to an altered growth fraction, i.e., a change in the number of cells actually traversing the cell cycle. This is best demonstrated by the occurrence of mitotic stimulation in the normally mitotically quiescent central zone of
the lens epithelium and the apparent broadening of the germinative zone as evidenced by the appearance of mitotic cells near the base of the meridional rows. The results of the $^3$H-Tdr experiments show that cells labeled in the germinative zone prior to the onset of inflammation do not contribute to the mitotic activity observed in the central and transitional zones. Although it is still possible that nonlabeled germinative zone cells may migrate, the rapid appearance of mitotic cells in the central zone makes this unlikely.

A proliferative increase in the lens epithelium associated with inflammation is not unprecedented. A similar mitotic stimulation of central zone cells has been reported as a sequela to inflammation resulting from cor-

Fig. 3. Micrographs of lens epithelial whole-mount preparations from a control eye and one injected with 0.2 μg of endotoxin 24 hr prior to sacrifice. A, Normal germinative zone, with the meridional rows in the upper portion of the micrograph. Note the large number of mitotic figures. B, Same area 24 hr after endotoxin injection is characterized by an absence of mitotic activity. C, Central zone of the normal epithelium shows little or no proliferation. D, There is considerable activity in the area following endotoxin administration. (×200.)
A mitotic stimulation in the lens due to an intraocular inflammation is consistent with recent findings\(^1\) that an intracameral injection of chemotoxic agents into the rabbit eye causes an increase in lenticular mitosis. The aqueous humor removed from eyes subjected to a multitude of other inflammatory agents\(^12\,13\) was found to be mitogenic when placed in vitro with rabbit lenses. This effect was attributed, at least in part, to the presence of serum protein in the aqueous humor following the breakdown of the blood-aqueous barrier (a normal sequela of intraocular inflammation)\(^14\) and the possible presence of inflammation-specific mitogens.\(^15\,16\) In the present study, such mitogens as well as a more direct synergistic effect of the endotoxin on the serum- and/or mitogen-stimulated lens epithelium cannot be excluded. In other systems, particularly cultured fibroblasts,\(^15\,16\) an enhancement of serum-stimulated DNA synthesis occurs in the presence of endotoxin.

The second inflammatory effect on cell division, the mitotic inhibition observed in the germinative zone, is difficult to explain, particularly since it occurs concomitant with the peak in mitotic activity in the central zone. A possible explanation might involve the influx of polymorphonuclear leukocytes (PMNs) which follows the injection of endotoxin. As previously reported,\(^8\) PMNs appear in the aqueous humor of the rat eye approximately 3 hr following endotoxin injection. By about 6 hr they begin to form a retroiridial hypopyon in the rat eye. The location of most of the inflammatory cells is therefore immediately over the germinative zone of the lens. Interestingly, the hypopyon begins to dissipate after 2 days, approximately the time that the mitotic increase begins in the germinative zone. Thus it may be that the PMNs are somehow interfering with the mitotic activity of the cells of that region. This is consonant with the observation that PMNs contain large quantities of prostaglandin \(E_1\)\(^17\) an au-
Fig. 5. Scatter plot of the mitotic activity in the transitional zone of the lens epithelium at intervals following the injection of 0.2 \( \mu \)g of Shigella endotoxin. The transitional zone, an area 10 cells wide lying between the germinative zone and the meridional rows, generally displays little or no mitotic activity. The scatter plot was derived from the whole-mount preparations used to generate the data in Fig. 2 (The symbols corresponding to those of Fig. 2 at the same intervals, indicate that the data were derived from the same preparation.) Note that the increase in mitotic activity in this region occurs concomitant with the mitotic overshoot in the germinative zone (Fig. 2, B).

Fig. 6. Time course of meridional row disorganization following the injection of endotoxin. Each point reflects the average disorganization of a single preparation, and the symbols at each interval correspond to the preparations used in Fig. 2. The disorganization is scored as 0 to 4 on the basis of the criteria outlined in the legend to Fig. 1. The dashed line traverses the means of individual points for each interval. Note the significant recovery of meridional row organization by 168 hr.

tocoid which has been shown to inhibit cell division in the lens in vitro.\(^{18}\)

The PMNs may also be responsible for the observed effect of intraocular inflammation on the alignment of the meridional row cells, since the equatorial location of the rows places them in the area of greatest PMN accumulation. The disorganization of the meridional rows within 12 hr of endotoxin administration suggests that the disorganization is being caused in cells which were in or at the base of the rows at the moment of endotoxin administration. This is supported by the fact that cells labeled in the germinative zone with \(^{3}H\)-Tdr at the time of endotoxin administration did not appear in the meridional rows at the height of meridional row disorganization (48 hr). Of particular interest is the observation that meridional rows appear to have undergone a significant degree of repair by the end of 1 week. A similar repair of endotoxin-induced meridional row disorganization also occurs in the frog lens (Rothstein and Hayden, personal communication). The degree of meridional row reorganization in the rat lens can be readily appreciated from an examination of Fig. 6. The rapid repair of meridional rows might explain why the endotoxin-induced inflammation failed to produce cataracts in animals examined up to 6 months after injection.

Since the time of Becker,\(^{19}\) who first coined the term "cataracta complicata," it has been known that inflammation can cause secondary or complicated cataracts. The fact that
Fig. 7. Photomicrographs showing the endotoxin-induced changes in the meridional row region when seen in sagittal section (A and C) and on whole mounts (B and D). A, Sagittal section of the bow region showing cells which appear swollen and somewhat disorganized in the area of the meridional rows 48 hr after endotoxin injection. Note the presence of PMNs in the extralenticular space (posterior chamber). B, PMNs can be seen as clumps of cells adherent to the capsule when viewed on a whole-mount preparation taken from a lens treated similarly. C, Sagittal section of a lens 7 days after endotoxin administration shows the return of the bow cells to normal and the elimination of the PMNs from the posterior chamber (although not shown here, occasional macrophages are still seen in the posterior chamber). D, Corresponding whole mount emphasizes the degree of reorganization of the meridional rows. (×200.)

cataracts did not appear in the present study may well be a result of the dose used. (The dose chosen for these investigations, 0.2 μg, was one which would produce a significant inflammatory response in the treated eye but have little consensual effect on the contralateral eye thereby allowing the use of the fellow eye as a control.) Experiments using higher doses have shown that an inflammation caused by injection of 20 μg of endotoxin would produce secondary cataracts within 5 months (Worgul and Merriam, unpublished results). It is likely, however, that the changes observed at the lower dose reflect the initial stages of events which occur at the higher levels.

Perhaps the most important observation in these studies is the remarkable similarity between the cytological effects of inflammation on the lens and those caused by other
cataractogens, most notably X-irradiation. In the case of radiation, however, there does exist a major difference in that although the meridional rows become disorganized following exposure to ionizing radiation, they do not return to normal.20, 21 As shown in the present report, meridional row disorganization caused by endotoxin-induced inflammation is reparable. Considering the previous findings which suggest a correlation between meridional row disorganization and cataractogenesis20, 22 it is of more than passing interest that cataracts caused by X-rays tend to be progressive but that complicated cataracts can be stationary, a situation which may reflect the reparability of the meridional rows.

The ability of the meridional rows to repair following endotoxin-induced inflammation intimates that the basis of this cytopathology may differ from that following X-irradiation. If such is the case, the role of inflammation in the pathogenesis of radiation cataract in the rabbit eye, as previously reported by this laboratory,23 may be one which is contributory and not causal.

REFERENCES