Often, one must wait for routine cultures to become positive before the diagnosis is confirmed. This commits the patient to potentially toxic antibiotic treatment, both systemic and intravitreal, pending culture results. It was the hope of this study that immediate measurement of vitreous glucose level would provide the clinician with a rapid means of differentiating bacterial from sterile inflammations when the diagnosis is uncertain. This, unfortunately, does not appear to be the case.

Vitreous glucose is depressed in endophthalmitis; markedly so with severe inflammation. This reduction occurs regardless of the etiology of the inflammation and, instead, is related to its severity. Therefore, vitreous glucose determination does not appear to be of clinical value in the differentiation of bacterial from sterile endophthalmitis.

Key words: bacterial endophthalmitis, ocular inflammation, intravitreal glucose, rabbits


References


Corneal Epithelial Cells Produce Thromboxane in Response to Interleukin 1 (IL-1)

Naveed B. K. Shams,* M. Michael Sigel,* † James F. Davis,* and James G. Ferguson†

Synthesis of thromboxane, a product of arachidonic acid formed via the cyclooxygenase pathway, was studied in rabbit corneal epithelial cells (SIRC cell line) under resting conditions and under the influence of interleukin 1 (IL-1). IL-1 potentiated the production of thromboxane 3-10-fold in a dose-dependent manner. This finding assumes added significance in view of the previous observations that the same cells are capable of producing IL-1. Thus, the corneal epithelial cells may be viewed in this context as an autocrine cell producing two biologically active substances which can serve as mediators of inflammation, one of which can augment the production of the other. Invest Ophthalmol Vis Sci 27: 1543-1545, 1986.

Rabbit corneal epithelial cells in primary culture, as well as an established line of rabbit corneal epithelial cells, produce a cytokine referred to as CETAF with properties characteristic of interleukin 1 (IL-1).1 2 We have confirmed the findings of Grabner et al by demonstrating that SIRC cell line produces an IL-1-like substance spontaneously and in increased amounts when stimulated by silica, LPS, lactic acid, or serum deprivation (Davis et al, unpublished). Thus, corneal epithelial cells may play an important role in local or regional physiological activities, which may be initiated and/or regulated by IL-1. Such activities include immunological reactions through the participation of lymphocytes, and histopathological effects such as injury and tissue repair. IL-1 can also increase the synthesis of prostaglandins and thromboxanes. It was previously reported that corneal cells are capable of synthesizing prostanooids.3 We, therefore, proceeded to determine whether production of these substances would be increased in corneal cells through the action of IL-1. Our findings indicate that SIRC corneal epithelial cells, which produce an IL-1-like substance, also respond to IL-1 by synthesizing increased amounts of thromboxane.

Materials and Methods. Cells and culture medium: Rabbit corneal epithelial cell line (SIRC) was obtained from American Type culture collection, Rockville,
In this study, we investigated the effect of IL-1 on SIRC corneal epithelial cells. Cultures were stimulated by various concentrations of IL-1. TXB2 cell line. 1.25 X 10^6 cells were seeded in 25 cm^2 flasks. Ninety-six hr after which time they were washed and fed with serum free RPMI 1640 medium supplemented with ingredients mentioned above. Some of the flasks received dilutions of IL-1, while others were given an equal amount of serum free growth medium. Flasks were incubated for 16-18 hr at 37°C in 5% CO2 incubator.

Interleukin 1: Human IL-1 prepared from acute monocyte leukemia cells was a gift from Dr. Lawrence B. Lachman of M. D. Anderson Cancer Center, Houston, Texas. This substance was highly active in the thymocyte proliferation assay.

Induction of thromboxane: Tissue culture flasks (25 cm^2) were seeded with 8 x 10^5-1 x 10^6 cells/flask. They were fed with 5 ml of culture medium for 96 hr, after which time they were washed and fed with serum free RPMI 1640 medium supplemented with ingredients mentioned above. The supernatants were harvested and subjected to radioimmunoassay (RIA) for thromboxane B2 (TXB2) (TxA2 is produced, but is unstable and is rapidly converted to TXB2).

RIA for TXB2: Anti-TXB2 antibody was kindly provided by Dr. Perry V. Halushka of the Medical University of South Carolina, Charleston. Cold TXB2 was obtained from Upjohn Diagnostics, Kalamazoo, MI. 3H-TXB2 was from New England Nuclear, Boston, MA. A working solution of this tracer was made in polyvinylpyrroliodine (PVP-40)-Tris-buffer (Sigma Chemicals, St. Louis, MO). The PVP-40 buffer contained Tris base, magnesium sulfate, calcium chloride, and sodium chloride, pH 7.4. The procedure measures the amount of cold TXB2 present in the supernatants of cells, by competition for a specific antibody with radioactive 3H-TXB2. The protocol was similar to that described by Burch et al., except for the use of PVP-40-Tris buffer and a different speed of centrifugation (2400 rpm for 20 min).

Statistical analysis: Using Data General’s Eclipse Computer, a standard curve was constructed from log/log using linear regression analysis. Correlation coefficient was deduced. Picogram levels of TXB2 were obtained from this curve. Each sample was run in duplicate in RIA for TXB2.

Results. Unstimulated SIRC cells produced thromboxane, and this synthetic process could be greatly augmented by the action of IL-1. In one experiment, the mean values for two unstimulated control cultures were 36.23 pg/100 μl and 45.14 pg/100 ml, compared to 475.56 and 608.65 for two cultures stimulated with 1:50 dil of IL-1. In another experiment, the corresponding values for unstimulated cells were 99.22 and 67.94, compared to 430.20 for cells stimulated with 1:50 dilution of IL-1 and 271.22 for cells stimulated with 1:100 dilution of IL-1. Correlation coefficient for each experiment was 0.998.

To further characterize the effect of IL-1 on SIRC corneal epithelial cells, we performed a dose-response experiment. The cultures were stimulated with increasing dilutions of IL-1, and end points were determined by RIA. Figure 1 shows that the action of IL-1 was dose dependent. The highest concentration (1/50) of IL-1 stimulated production of 618.45 pg of TXB2 per 100 μl of sample. At the end point, which fell between 1/800 and 1/1600, IL-1 activated cells synthesized 84 pg of TXB2. Supernatants from unstimulated cells had baseline levels of 37.86 pg and 48.43 pg of TXB2. Serum free RPMI 1640 medium showed approximately 3 pg/100 μl TXB2, which is insignificant in these assays.}

Discussion. Our study demonstrates that corneal epithelial cells in culture respond to IL-1 with an increased production of thromboxane. These findings assume added significance, in view of the previous evidence that the same cells are capable of producing IL-1. Both are potent mediators of inflammation, and the fact that both can be synthesized by the same cell and that IL-1 can augment the production of thromboxane endows this cell with an autoregulatory capability. IL-1 has been shown to participate in numerous physiological, immunological, and inflammatory events. These include fever, acute phase response, lymphocyte proliferation, enhanced expression of receptors for IL-2, fibroblast proliferation, and muscle degradation. Some of these events are caused by IL-1, while others result from the...
action of prostanoids whose formation is augmented by IL-1. However, considerable controversy exists with regard to pinpointing the exact mediator of some of these events.

To our knowledge, this is the first report of IL-1 inducing increased production of thromboxane in corneal epithelial cells, but there already exists considerable evidence that ocular tissues possess enzymes capable of metabolizing arachidonic acid leading to the production of prostaglandins and thromboxanes.\textsuperscript{8,9} Recent studies on the rabbit cornea demonstrated that there was a differential formation of prostaglandins, thromboxane, and hydroxyeicosatetraenoic (HETE) in response to cryogenic trauma.\textsuperscript{10} Separated layers of the cornea, i.e., epithelium, stroma, and endothelium, produced different amounts of products of arachidonic acid derived by the cyclooxygenase and lipoxygenase pathway. The authors attributed the changes, at least in part, to the migration of inflammatory cells, as these are known to synthesize various mediators. There is no doubt that monocytes/macrophages, lymphocytes, and polymorphonuclear leukocytes contribute substantial amounts of these mediators, but it now appears that epithelial cells are also active in this respect.

A study on separated cells of rabbit corneas disclosed that unstimulated epithelial cells produced a substantial amount of thromboxane, a moderate amount of PGF\textsubscript{2\alpha}, and a small amount of 6-keto PGF\textsubscript{1\alpha}.\textsuperscript{3} In contrast, corneal fibroblast of rabbit and calf were committed to synthesis of PGE\textsubscript{2}, and produced no detectable thromboxane. No information was given about attempts to increase production of thromboxane in epithelial cells.\textsuperscript{3} Our findings confirm and extend the role of corneal epithelial cells in this regard and, in fact, add new evidences that the cell may function as an autocrine cell by producing both IL-1 and thromboxane, with the former having the capability of stimulating the production of the latter.

Based on a priori evidence in other systems, IL-1 can augment the production of various prostanoids and it is, therefore, possible that IL-1 synthesized by corneal cells may stimulate increased production of prostaglandins by corneal fibroblasts. This point needs to be ascertained. It is also very important to stress that these substances can interact antagonistically or protagonistically with respect to chemotaxis, edema, or immunoregulation.\textsuperscript{11}

Key words: interleukin-1, thromboxane, SIRC cells line, prostanoids, cytokine

Acknowledgment. The authors are grateful to Dr. Ronald Beck, Department of Physiology, University of South School of Medicine, Columbia, South Carolina for providing a program for radioimmunoassay and statistical analysis.

From the *Department of Microbiology and Immunology and the \textdagger Department of Ophthalmology, University of South Carolina School of Medicine, Columbia, South Carolina. Submitted for publication: December 26, 1985. Reprint requests: M. Michael Sigel, Department of Microbiology and Immunology and Department of Ophthalmology, University of South Carolina, School of Medicine, Columbia, SC 29208.

References