Identification of inflammatory mediators in proliterative retinal diseases

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A. Scientific Abstract

Proliferative retinal diseases are often complicated by the formation of retinal membranes that can contract and result in retinal detachment. Two such diseases are proliferative diabetic retinopathy (PDR) and proliferative vitreoretinopathy (PVR), the most common cause of retinal reattachment surgery failure.

PDR and PVR share common features in the pathogenesis of retinal membranes. Briefly, a response to injury---either to the retina in PVR or to microvasculature in PDR-triggers the migration, proliferation, and differentiation of various celis. This ultimately results in the formation of collagenous membranes that can contract and impart tractional forces to induce breaks or detachment (1,2). While surgical retinal reattachment is possible, a significant number of patients experience postsurgical recurrence of disease. Also, there is no pharmacotherapy for these diseases, and only one drug is currently in clinical trials for the prevention of recurrent PVR.

It is hypothesized that common cytokines, cell adhesion factors, and other inflammatory mediators play roles in the pathogenesis of these proliferative retinal membranes. While many of these have been implicated or identified, their specific roles have yet to be elucidated. Identifying and defining the role of these inflammatory mediators may lay the groundwork for future research to design novel therapies for these diseases.

We plan to continue our study of these potential pathogenic factors by examination of surgical vitreous samples for the presence of selected inflammatory markers.

B. Lay Abstract

In proliferative retinal diseases, such as proliferative vitreoretinopathy (PVR) and proliferative diabetic retinopathy (PDR), scar tissue, or membranes, may form on the retina of the eye and complicate the repair of retinal detachment. If this occurs, the patient undergoes surgery to remove these membranes to reattach the retina.

ne goal of this research is to identify and define the role that certain molecules play in causing the growth of these membranes. To do so, we will ask patients to allow us to keep any vitreous fluid that is removed from their eye during their surgery. (I'his fluid is normally removed and discarded as part of their surgical procedure.) We will then look for the presence of these molecules in the vitreous sample with assays.

C. StudyDescription

a. Study Purpose and Rationale

Proliferative retinal diseases are often complicated by the formation of retinal membranes that can contract and result in retinal detachment. Two such diseases are proliferative diabetic retinopathy (PDR) and proliferative vitreoretinopathy (PVR), the most common cause of failure of retinal reattachment surgery.

Although PDR and PVR are distinct clinical and pathological diseases, they share some common features in the pathogenesis of retinal membranes. It is thought that in PVR, an initial retinal injury triggers the breakdown of the blood-retinal barrier. Abnormal levels of serum and locally-produced cytokines then mediate the migration and proliferation of various cells that result in an exaggerated

wound-healing response. The proliferating cells are induced to differentiate into myofibroblast-like cells that then form collagenous membranes. Contraction of these epiretinal membranes imparts tractional forces that can reopen the intial tear, cause new breaks, or induce detachment (1). A similar process occurs in PDR, in which an angiogenic response to diabetic microvascular injury leads to the recruitment of endothelial cells and the formation of new extraretinal vessels with a fibrous component. Molecular inflammatory mechanisms may also contribute to this neovascularization. Regression of these vessels leaves residual fibrovascular proliferation along the posterior hyaloid and that can then impart tractional forces on the vitreous (2).

Anatomical reattachment of the retina is possible with modern vitreoretinal surgical techniques that remove epiretinal membranes and relieve tractional tension. However, a significant number of patients experience postsurgical recurrence of disease in the form of retinal detachment, new breaks, or new proliferation. Also, there are no pharmacologic interventions for these diseases; currently, there is one drug in clinical trials for the prevention of recurrent PVR, but it appears unlikely that targeting therapy to one of these inflammatory mediators will stop the entire cascade of cellular events that form tractional retinal membranes. Instead, multiple agents directed at different stages of the process -- a strategy analogous to chemotherapy for cancer-may be more successful in promoting visual outcomes in patients.

Our current work has identified the presence of some of the mediators that may be involved in the events leading to the abnormal wound-healing response seen in these proliferative retinal disorders. However, the specific role of each of these mediators has yet to be elucidated. Correlation of the plasma level of these mediators with their levels in the vitreous is also needed to better define their roles in PVR and PDR. These studies may lay the groundwork for future research to design novel therapies for these proliferative retinal diseases.

b. Background

In patients with PVR and PDR, both monocyte-derived macrophages and lymphocytes have been identified in surgically removed fibrovascular and fibrocellular membranes (1). Recruitment of these cells to sites of inflammation requires their adhesion to vascular endothelium and subsequent migration into surrounding tissue. Many inflammatory mediators mediate this process, and those that have been implicated in the pathogenesis of epiretinal membranes in PVR and PDR include:

i. Growth factors

Many growth factors have been implicated in promoting neovascularization and fibrosis in the eye. The best-characterized angiogenic growth factor in ocular neovascularization is vascular endothelial growth factor (VEGF) (2,3), an angigogenic mitogen that also promotes vascular hyperpermeability. Another growth factor that has demonstrated involvement is platelet-derived growth factor (PDGF) (4), which stimulates processes such as fibroblast and smooth muscle proliferation and migration.

ii. RAGE axis

Also implicated is the proinflammatory RAGE axis, seen in numerous diabetic complications. AGEs (advanced diabetic glycation endproducts) are compounds that are found in plasma and vessel walls in normal aging, but accumulate in an accelerated degree in diabetic patients. The interaction of AGEs with its receptor (RAGE) stimulates the expression of cellular adhesion molecules. RAGE has been found to have other ligands; for example, it serves a signal transduction receptor for S100/calgranulin-like molecules, a family of polypeptides released from activated inflammatory cells. Their interaction is believed to have a role in sustaining inflammatory response in a variety of chronic disorders. Our preliminary studies have indicated a role for the RAGE axis in the pathogenesis of retinal proliferative diseases (5,6).

c. Study Design and Statistical Procedures

Levels of inflammatory mediators in vitreous fluid and blood will be noted as concentration per millimeter of fluid. Data will be analyzed by one-way analysis of variance and one-tailed t-tests will be performed. Mean concentrations of inflammatory mediators will be comparedwith mean concentrations in controls. A p-value < 0.05 will be considered significant for all tests. Statistical software will be used to perform all calculations. Preliminary data in both human and experimental models of proliferative retinal

diseases indicates that 30% increase in levels of inflammatory mediators is found to be significant. Data also suggests that standard deviation of up to 50% of mean levels can be found. An unpaired t-test comparing the means in control and uncontrolled cases indicates that 46 patients will be need in each group to detect a significant difference between the groups.

d. Study Procedures

i. Clinical specimens

The study protocol will enroll patients who plan to undergo vitrectomy surgery with retinal detachment due to PVR or PDR. Patients undergoing surgery for repair of macular holes will also be recruited as controls for vitreous specimens. Informed consent will be obtained from each patient for analysis of a blood sample, to be collected at the time of intravenous catheter insertion just prior to surgery. The sample will be centrifuged at 4000 rpm, and the cell-free plasma supernatant aspirated for storage at -80 degrees C. Vitreous fluid samples will be collected during the surgical procedure via vitrectorny probe and transferred to heparinized tubes and aliquoted for storage at -80 degrees C. It is anticipated that an enrollment period of 24 months will be necessary for an adequate collection specimen samples.

ii. Assays

Vitreous and plasma will be assayed for VEGF and PDGF. The assays will be performed using commercially available ELISA kits according to manufacturer's instruction. In additions, ELISAs developed in the laboratory of Dr. Ann Marie Schmidt at the Columbia University Department of Surgery (a collaborator of these studies) will be used to study the levels of AGEs, other RAGE ligands including S-100 calgranulins, and the levels of soluble RAGE.

Assays will be performed at various intervals (every three to four months) as specimens are collected. This allows us to identify and correct any problems we may encounter with methodology.

D. Study Drugs or Devices

N/A

E. Study Questionnaires

N/A

F. Study Subjects

a. Inclusion Criteria

- Patients with a macular hole or recurrent epiretinal membranes as a result of proliferative retinal disease scheduled for vitrectorny surgery.
- Willingness to participate and sign informed consent

b. Exclusion criteria

- Other ocular disease (e.g. vascular occlusion, uveitis, significant vascular hemorrhage) that may influence inflammatory findings.
- Silicone oil already present in the eye.
- Minors (patients under the age of 18).

G. Recruitment of Subjects

Subjects of this study will be recruited from patients who have surgical indications for undergoing vitrectomy. They will be identified and approached by their treating surgeon. They will have already provided informed consent for the proposed surgical procedure, which includes vitreous and epiretinal membrane removal. They will then be approached about the study and providing a blood ample on the day of the surgery.

After being informed that participation is voluntary, and that they may withdraw from this study at any time without prejudice, the patient will date and sign the IRB-approved consent form in the presence of a witness. This will enroll them in this study.

No minors will be enrolled in this study. Non-English speakers will be accepted into the study only if a reliable translator is available.

H. Confidentiality of Study Data

Data and specimens from each patient will be coded with a unique identification number. This information will then be encrypted and stored in a computerized database to ensure strict confidentiality for the patient. Tbis database will not be accessible to any third party.

I. Potential Risks

The removal of vitreous fluid is a routine part of the surgical procedure; it is normally discarded after removal from the eye. Its collection and use in this study, therefore, constitutes no added risk or discomfort to the patient.

J. Potential Benefits

The patient may or may not benefit personally from this study.

Surgical management of these disorders can be improved by 1) an understanding of anatomical and surgical risk factors that predispose patients to recurrence; and 2) delineation of the inflammatory mediators and their roles in the pathogenesis of tractional epiretinal membranes. Also, there are no approved drugs for the treatment of these recurrent diseases, and only one currently in clinical trials (for the prevention of recurrent PVR). Results of this study may aid in the development of novel pharmacotherapics for these conditions. Therefore, benefits to society may include the improvement of therapeutic management of these potentially blinding proliferative retinal diseases.

The study requires additional procedures to the patient's care that are minimally invasive; they pose little to no additional risks for the patient. It is felt that this risk is reasonable in light of the potential anticipated benefits and knowledge that may be expected from this work.

K. Alternatives

As this study is not considered therapeutic, there are no alternatives to participation other than not participating. Patients who choose not to participate will continue to receive the standard of care for their conditions.

L. Litetature Citcd

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