Clinical experiments in cellular immunity in eye disease

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Experiments with leukocyte migration inhibition (LMI) in various ocular disorders indicate sensitivity to cornea and lens most frequently, and to retina, choroid, and iris less often. The immunologist's dictum that sensitivity develops to "foreign" proteins is borne out since cornea and lens are relatively, if not completely, avascular after birth. Herpes virus infection of the cornea not only induces vascularity but exposes the lymphocyte to a cell not previously known to it and possibly transformed by viral DNA. The release of lymphokines such as lymphotoxin may increase pathology. Other mediators of cellular immunity (blastogenic, migration inhibitory, and leukotactic factor) may assist healing by "walling" the antigen in a granuloma as in tuberculosis. It is not known which mediator is of greater importance in viral keratitis, toxoplasmic chorioretinitis, and retinal detachment. Lens protein appears not to enter the circulation after cataract extraction. Severe inflammation adjacent to the lens may sensitize the lymphocyte to lens protein, lead to lymphotoxin secretion, and enhance lens damage. Other in vitro tests must be used to study cell-mediated immunity since LMI has shown that it is involved in many ophthalmologic conditions.

Key words: leukocyte migration inhibition (LMI), lymphokines, lymphocyte stimulation, lymphocyte transformation, blastogenic factor, leukotactic factor, lymphotoxin, cellular transformation.

Cellular immunity is being investigated in disorders affecting the kidney, the central nervous and other systems, and autoimmune disorders. Its role in the pathophysiology of eye disease has not been studied sufficiently in view of its potential importance and the ease of observation of ocular disorder. The lymphocytes are the mediators of cellular immunity. The clonal selection theory and current thought postulate that the lymphocyte has antigen-recognizing receptors on its membrane. When this cell encounters foreign molecules, it is sensitized and stimulated to divide and give rise to two populations of cells, the ones manufacturing antibody, the others mediating cellular immunity. The process can be regarded as having an afferent arc of lymphocyte sensitization and an efferent arc with cell activation, antibody secretion, and release of the mediators of cell-mediated immunity, the lymphokines. An increasing number of lymphokines is being reported, but most clinical studies...
have been performed with the lymphocyte stimulation or transformation factor, the migration inhibitory factor, and lymphotoxin, all of which may be multiple factors.

**Lymphocyte transformation and stimulation**

Lymphocyte transformation represents a change in cell morphology causing it to resemble the atypical cell of infectious mononucleosis. It has been observed in two of four patients with sympathetic ophthalmia with 0.1 mg. of protein from lens and retina used as antigen. A preparation of uveal-retinal tissue containing six antigenic components in 10 mg. per milliliter produced significant lymphocyte transformation in five of six patients with sympathetic ophthalmia when the investigators utilized 1,000 lambda. Non soluble bovine uveal pigment in a dosage of 2 mg per milliliter stimulated lymphocytes to incorporate tritiated thymidine in two patients with the Vogt-Koyanagi-Harada syndrome and in three patients with sympathetic ophthalmia.

Lymphocyte transformation was assessed by protein incorporation of C-14 in patients with herpetic keratitis; corneal antigen doses ranging from 0.1 to 1.0 mg of soluble protein did not produce increased protein synthesis. Subsequent experiments confirmed these results utilizing tritiated thymidine to evaluate DNA synthesis. In six patients with miscellaneous ocular disorders: sympathetic ophthalmia, retinitis pigmentosa, post-cataract extraction, and uveitis, antigens prepared from soluble protein extracts of cornea, ciliary body, sclera, lens, and retina caused no increased protein synthesis. The small number of patients and the fact that they were not all tested with all relevant antigens permit no definite conclusion. In another study, two of thirteen patients' own aqueous humor produced lymphocyte transformation in cases of uveitis of diverse origin. The clinical condition of the patients, and the source and solubility of the antigens are varied; moreover, the inflammation of uveitis may transport the patient's immunoglobulin into the aqueous humor in a concentration sufficient to cause transformation. These investigations cannot be compared because of the differences in antigen, some being a combination of tissues, both homologous and heterologous. Lymphocyte transformation should be named lymphocyte stimulation and measured by incorporation of a radioactive precursor, which is an objective indicator of cell-mediated immunity, while transformation is a subjective evaluation. More objective studies are needed in the study of cellular immunity in eye disease.

**Migration inhibition.** Migration inhibition can be studied by incubating the patient's lymphocytes with the suspected antigen and adding the supernate to a population of guinea pig macrophages. Cell-mediated immunity is demonstrated if the supernate leads to macrophage migration inhibition. A more direct method packs human leukocytes into capillary tubes in the same manner as the above guinea pig macrophages and allows them to migrate into medium-containing chambers. The suspected antigen is added to the medium and cellular immunity is demonstrated if leukocyte migration inhibition (LMI) of 20 per cent or more takes place. A variety of ophthalmologic disorders have been studied by the human leukocyte migration method. It was found that soluble protein from cornea in a concentration of 0.2 and 0.1 mg. per milliliter induced LMI in 14 of the 16 patients with chronic keratitis.

In a greatly expanded study, keratopathies associated with increased vascularity generally demonstrated LMI by corneal antigen while nonvascularized corneal opacities showed LMI infrequently.

Cell-mediated immunity was studied in 40 patients who had anterior chamber surgery and cataract extraction. Cornea, iris, and sclera are cut during the operation. LMI was detected in one-third of patients by cornea, one-quarter of patients by iris, and in two of nineteen patients by sclera. Patients with sensitivity to any tissue usually had a history of prior anterior chamber inflammation frequently detected before surgery. The surgical procedure itself rarely produced cell-mediated immunity. There was no LMI by ciliary body in 15 patients studied pre- and/or postoperatively. These findings were corroborated by patients with glaucoma who were sensitive to the same tissues more often when there was associated disease.

Lens protein was tested in over 60 patients with cataracts, increased intraocular pressure, operations for the two previous disorders, diabetic retinopathy, and uveitis. LMI was detected in 11 of 13 patients with cataracts and in one-quarter of a similar number of patients without cataract, but with a history of anterior uveitis, associated glaucoma, or prior surgery for relief of increased intraocular pressure. Only one of fifteen patients developed sensitivity to lens after simple cataract extraction.

Retina and choroid were tested in 38 experiments in 22 patients with diabetic retinopathy before and after numerous photocoagulation applications. Retina and choroid produced LMI in six and three patients, respectively; only once before photocoagulation therapy, yet sensitivity usually did not persist. Prolonged un repaired or repaired retinal detachment led to LMI with retina in six of nine patients and with choroid in four of eight patients, while there was no LMI by either tissue if detachment had lasted.
Table 1. Significant LMI* in disorders of the eye

<table>
<thead>
<tr>
<th>Antigens 0.2 mg. protein per milliliter</th>
<th>Cornea</th>
<th>Iris</th>
<th>Ciliary body</th>
<th>Sclera</th>
<th>Lens</th>
<th>Choroid</th>
<th>Retina</th>
<th>Uvea</th>
<th>Optic nerve</th>
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<tbody>
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<td>Acute keratopathy†</td>
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<tr>
<td>Chronic active keratitis</td>
<td>15/25</td>
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<td>Scarred nonvascularized cornea</td>
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<tr>
<td>Glaucoma</td>
<td>8/22</td>
<td>5/17</td>
<td>0/14</td>
<td>2/5</td>
<td>1/7</td>
<td>1/15</td>
<td>1/8</td>
<td>1/7</td>
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<tr>
<td>Cataracts</td>
<td>5/13</td>
<td>0/8</td>
<td>0/7</td>
<td>0/3</td>
<td>5/14</td>
<td>6/13</td>
<td>2/9</td>
<td>3/9</td>
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<tr>
<td>Cataract extraction</td>
<td>8/27</td>
<td>4/19</td>
<td>0/9</td>
<td>2/15</td>
<td>7/26</td>
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<td>0/7</td>
<td>0/4</td>
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<td>Anterior uveitis</td>
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<td>1/6</td>
<td>0/2</td>
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<td>2/7</td>
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<td>0/2</td>
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<td>3/12</td>
<td>3/7</td>
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<td>1/8</td>
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<td>Diabetic retinopathy</td>
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<td>Prolonged retinal detachment†</td>
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<td>Macular degeneration</td>
<td>0/3</td>
<td>0/3</td>
<td>0/2</td>
<td>0/3</td>
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<tr>
<td>Cellular immunity to homologous tissue</td>
<td>63/165</td>
<td>16/89</td>
<td>1/56</td>
<td>7/45</td>
<td>22/77</td>
<td>33/157</td>
<td>42/143</td>
<td>19/61</td>
<td>2/5</td>
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</table>

*Patients with leukocyte migration inhibition greater than 25 per cent shown in fraction form over number of patients tested with each tissue. In diabetic retinopathy, the fraction indicates experiment numbers not patient numbers.
†Within one month of infection or injury.

One month or less. Eight of fifteen patients with healed chorioretinal disease had LMI by retina, as did eight of thirteen patients with active chorioretinitis. Sensitivity to choroid was found in three patients with inactive disease and in six patients with active inflammation.

Senile macular degeneration was not associated with LMI by choroid or retina in six patients. The cells of eight patients with optic atrophy and neuropathy of diverse etiology were also tested with optic nerve antigen. A six-month-old baby had marked LMI by optic nerve and by choroid and retina; two other patients had LMI by optic nerve of borderline significance. Three patients with Best’s vitelliform degeneration were investigated; one of them had LMI by choroid. There was no evidence of cellular immunity in two brothers or their father, while the mother with pigmented retinal lesions had LMI by retina.

Protein from all the tissues of uveal tract when used as antigen in a concentration of 1 mg. per milliliter produced LMI in eleven of twelve patients with uveitis. When the concentration of uveal tract protein was 0.25 mg. per milliliter, LMI was noted in seven of the twelve patients. Another group of 25 patients with peripheral and diffuse uveitis was tested with individual homologous uveal tract and other ocular tissues in a concentration of 0.2 mg. protein per milliliter and LMI was found less frequently. Cornea produced LMI in slightly less than half of the patients, iris and choroid in one-fifth of the patients, and retina in one-third of the patients. Ninety-three per cent of patients with Sjögren’s syndrome had LMI with parotid extract in the dose of 0.2 mg. per milliliter. Studies with etiologic agents. Various dilutions of live herpes simplex and varicella virus did not produce lymphocyte stimulation or LMI. Larger doses, in terms of protein concentration of noninfectious herpes simplex antigen produced lymphocyte stimulation of cells from sensitized animals and patients with viral keratitis.

The different results obtained in various laboratories with lymphocyte stimulation by herpes simplex can be ascribed to difference in antigen, i.e., live virus or complement-fixing antigen, different antigen dosages, and experimental conditions.

Dilutions of toxoplasma trophozoites treated with 1 per cent formalin and purified, caused LMI in patients with toxoplasma chorioretinitis. Tests are in progress to evaluate diverse toxoplasma antigens in a study of cellular immunity and its relationship to disease activity. Experiments relating toxoplasma serology (Sabin-Feldman dye test and fluorescent IgM antibody) with cell-mediated immunity (lymphocyte transformation, human leukocyte and guinea pig macrophage migration inhibition) have been reported. Lymphotoxin. There have been no publications regarding lymphotoxin in eye disease in the immunologic or opthalmologic literature. Lymphocyte stimulation, macrophage migration inhibition, and lymphocyte cytotoxicity to viral antigen and
to its complement-fixing antibody gave different results.29 Migration inhibition and cytotoxicity were both impaired in patients whose lymphocytes were challenged with viral antigen. There was no correlation with the patients' complement-fixing antibody,29 as was reported in toxoplasmosis. Lymphotoxin is being sought in diseases in which LMI was most significant.14

Corneal graft and immunosuppression. Corneal graft rejection was reviewed in these pages two years ago.30 Graft rejection is considered a prime example of cell-mediated immunity and anti-lymphocytic serum may be used to delay the rejection syndrome.31,32 Immunosuppression has been evaluated in sympathetic ophthalmia and other ocular inflammatory diseases.33 The anti-inflammatory and antimetabolite agents depress cellular immunity in the host; they interfere with the in vitro tests, which do not respond similarly.34

Conclusion

The LMI experiments suggest that cells and proteins to which the patients' lymphocytes are not normally exposed are not recognized as "self" and induce cellular immunity. Corneal vascularity becomes extensive with inflammation due to trauma or infection. The etiologic agent, e.g., herpes virus in keratitis followed by anterior uveitis and toxoplasmosis causing chorioretinitis, both intracellular parasites, leads to cell membrane changes which stimulate the cellular immune system. Antigen-antibody reactions produce vasculitis in the uveal tract and/or antigen-antibody complexes combined with complement lead to vascular obstruction. Thus, humoral immunity or intense inflammation itself may alter known tissues including vascular ones and render them antigenic. The transfer of uveitis with cells but not with serum from afflicted to healthy guinea pigs indicates that cellular immunity plays a fundamental role in the pathophysiology of uveitis.35,36

Lens protein appears "unknown" to the lymphocyte and sensitizes it on first encounter, usually during inflammation in adjacent tissue, rarely after routine cataract extraction, and occasionally when there is degeneration. Retinal pigment appears to represent transformed antigenic tissue and can be associated with cellular immunity as opposed to a senile cataract.

Vascular insufficiency in senile macular degeneration, diabetic retinopathy, and glaucoma reduces the exposure of the lymphocyte to potentially antigenic tissue and decreases the opportunity of lymphokine secretion. Some lymphokines (blastogenic and leukotactic factors) assist the body's attempt to repair pathology as in retinal detachment, while lymphotoxin may be partly responsible for chronicity in infections due to herpes virus hominis and toxoplasmosis.

The earlier observations of ease of establishment of long-term culture of lymphoid cell lines with 25 ml. of blood in chronic keratitis, suggested systemic involvement in localized disease.36,37 LMI with homologous tissue antigen confirms the importance of cell-mediated immunity in ophthalmologic disorders and the need for complete investigation (Table I).

The ability and patience of Miss Barbara Hood have allowed this review of experiments and literature.

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