Anterior chamber lymph node implantation: Local adoptive immune response in the eye

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The rabbit anterior chamber was used for the implantation of specifically sensitized autologous lymph node tissue, and the response to local and systemic injection of antigen studied. Clinical observation of the sensitized eye following challenge with specific antigen showed a marked hemorrhagic iritis with typical anterior chamber "flare" and fibrous exudate. This response occurred within hours after local antigenic challenge, reached a peak within 24 to 48 hours, and gradually subsided over a period of 2 to 3 weeks. Microscopic examination revealed marked cellular activity within the implanted lymphoid tissue and a striking increase in the number of plasma cells in the iris and corneal stroma, most marked at the limbus. These results are most consistent with the view that immunologically competent cells within the eye respond to local or systemic antigenic stimulation resulting in clinical uveitis. It is felt that this experimental model may provide a useful tool for further investigation of a wide spectrum of uveitis-related mechanisms.

The technique of transplanting various tissues to sites within the eye has been practiced for many years and survival of small pieces of donor tissue amply confirmed. Previous investigations concerning such implants within the anterior chamber have been directed primarily to the physiology and functional behavior of the donor tissue. The production of thyroid hormone, the observation of an hormonal control of ovulation, and the growth patterns of malignant tissues have been well described.

Silverstein found that the eye itself may act (in some degree) as if it were a lymph node when secondarily stimulated with antigen. The ocular response to such stimulation was found to consist of a marked inflammatory infiltration of the uveal tract with proliferation of lymphoid elements including plasma cells. He thus demonstrated a resemblance between nongranulomatous uveitis and the anamnestic response seen in eyes previously injected with antigenic material.

The behavior of lymph node tissue in the anterior chamber has not previously been reported. The use of autologous lymph node implants permits the introduction of cells known to be immunologically competent into the anterior chamber of an eye not previously exposed to antigen. The present studies were undertaken in an effort to clarify the cellular events and mechanisms involved.
clinical uveitis which followed stimulation of quiescent but immunologically prepared tissue within the anterior chamber. It has been possible to demonstrate the marked resemblance between the anamnestic response of the implanted lymphoid tissue and the previously reported responses to secondary stimulation of the eye following primary intravitreal inoculation of antigen.\textsuperscript{7}

**Materials and methods**

Albino rabbits of both sexes weighing between 3 and 5 Kg. were initially sensitized by a single injection of 1 mg. of bovine serum albumin, ovalbumin, or human gamma globulin (pooled human fraction II) in complete Freund's adjuvant placed in the subcutaneous tissues of the ear to stimulate primarily the draining pre-auricular lymph node. After 7 to 14 days this lymph node was removed, cut into small pieces 1 to 3 mm. in diameter, and one or two of these fragments placed in the anterior chamber of the same animal. In all cases autologous transplants were made in order to obviate the possibility of homograft reaction on the part of the host complicating subsequent observations. The eye was then observed until all evidence of inflammation had subsided (4 to 7 days).

In the major series, 6 animals having lymphoid implants in only one eye were challenged with 50 gamma of homologous antigen injected intracorneally (Series 1), and 10 others, with implants in both eyes, were challenged with homologous antigen in one cornea and unrelated antigen in the other (Series 2). Other rabbits with sensitized lymphoid tissue in one eye were subsequently challenged with 5 mg. of homologous antigen intravenously. Animals bearing nonsensitized or adjuvant sensitized anterior chamber implants were challenged intracorneally with several antigens. Injections were done 8 to 15 days after lymph node implantation.

The animals were observed clinically with the Zeiss slit lamp for pertinent observations prior to and after challenge. After periods of 2 to 19 days the animals were sacrificed and the eyes removed for fixation in alcohol-formalin. Residual lymph node tissue was similarly treated at the time of transplantation. Hematoxylin and eosin as well as methyl green pyronine stains were routinely performed for histologic evaluation of the lymph node tissue and of both eyes in all animals.

**Results**

Slit lamp examination of all eyes following transplantation of lymphoid tissue exhibited mild injection of the conjunctiva and iris for 1 to 3 days. In all cases evidence of inflammation subsided within 7 days, at which time the implanted tissue was seen to be vascularized and firmly attached to the underlying iris (Fig. 1). Subsequent observations showed continued viability of nonstimulated implants (up to 6 months) and no further evidence of ocular inflammation.

Following intracorneal injection of homologous antigen, all eyes containing pre-

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*Fig. 1.* Rabbit eye showing quiescent anterior chamber with lymph node implants on the iris at 5 and 9 o'clock. Note the easily discernible vessels supplying each lymph node fragment.

*Fig. 2.* Rabbit eye showing marked anterior uveitis and corneal clouding 4 days after intracorneal injection with 50 gamma of homologous antigen. Prominent vascular dilatation at the limbus is evident.
viously sensitized lymphoid tissue exhibited a similar reaction. After 12 to 24 hours there was severe photophobia and chemosis; the iris became engorged and peripheral iris vessels were prominent. At 24 hours there was marked anterior chamber "flare" and moderate to marked proteinaceous precipitate. By 36 hours engorgement of the implanted lymph node with dilated vessels was seen on the anterior iris about the implant. In many instances the implants themselves became reddish in color and exhibited similar injection within their substance. The cornea of each eye exhibited a hazy ring, not unlike a Wessely ring in appearance, except that it was somewhat wider and less discrete. The cornea within the ring was slightly hazy and thickened. Both the conjunctival chemosis and corneal ring began to diminish after 36 hours. However, the engorgement of iris and lymph node tissue reached a peak at about 48 hours and continued to be the most prominent clinical feature of the reaction (Fig. 2). Corneal and conjunctival changes completely subsided at the end of a week, whereas the iris and lymph node engorgement persisted for at least 2 weeks. The "flare" and anterior chamber precipitate resolved within 10 days.

Rabbits bearing sensitized lymphoid tissue implants in one eye only and subsequently challenged intravenously with 5 mg. of the homologous antigen developed clinically apparent uveitis and anterior chamber inflammation only in that eye. Although similar to the reaction of homologous intracorneal injection of antigen described above, the response was much less marked in this series of animals. Intracorneal injection of unrelated antigen in eyes containing previously sensitized or normal lymphoid tissue produced no inflammatory response. These clinical observations are summarized in Table I.

As the clinical evolution of these reactions was observed animals were sacrificed at various intervals for histologic examination. The microscopic appearance of non-stimulated eyes examined shortly after lymph node implantations showed only a mild polymorphonuclear response confined to the limbus and peripheral cornea. The

### Table I. Effect of antigenic stimulus following lymph node implantation

<table>
<thead>
<tr>
<th>No. animals</th>
<th>Lymph node implant</th>
<th>Primary sensitizations</th>
<th>Secondary stimulation</th>
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Fig. 3. Anterior chamber implant in an unchallenged eye. The lymph node shows minimal proliferative activity and the boundary between the implant and iris stroma is well defined. (H & E. Original magnification, x80.)

Fig. 4. Anterior chamber lymph node implant 5 days after intracorneal injection with homologous antigen. Note the marked vascularity of the iris and the cellular infiltrate in the iris stroma. There is also a thin clot of fibrin and intermeshed mononuclear cells adjacent to the iris in the anterior chamber. (H & E. Original magnification, x40.)

Lymph node tissue maintained its general integrity with no evidence of necrosis or unusual cellular proliferation. Small secondary follicles and occasional vacuoles surrounded by epithelioid and giant cells were present (Fig. 3). These features were similar to those seen in sections of the same pre-auricular lymph node from which the implants were taken. Eyes bearing presensitized lymphoid tissue implants were quite similar in appearance following intracorneal stimulation with unrelated antigen, exhibiting quiescent, well-demarcated lymphoid tissue with only scattered remnants of the reparative inflammatory infiltrate. The iris stroma and vasculature were entirely normal.

In marked contrast, eyes challenged intracorneally with homologous antigen showed intense cellular activity both within the implant and surrounding iris stroma as well as the cornea. The lymph node fragments showed much more intense secondary follicle formation with prominent mitotic and phagocytic activity (Figs. 4 and 5). The boundary between the follicles and the surrounding zone of small lymphocytes was less discrete. In addition, the line of demarcation between the implant and surrounding iris connective tissue was partially obliterated. Large numbers of lymphocytes and plasma cells apparently invading the surrounding stroma could be found in the most reactive examples, as seen in Fig. 6. Distant from the implant, the stroma of the iris and ciliary body was infiltrated with mononuclear cells, including numerous large pyroninophilic cells and well-developed members of the plasma cell series (Fig. 7). Free lymphoid cells, including plasma cells and macrophages, were frequently seen within the anterior chamber, as well as occasional fibrin deposition about adjacent structures. In addition, lymphocytes and plasma cells were also present between the corneal lamellae with scattered polymorphs in the zone of the hazy ring seen on slit lamp examination.

As the reaction regressed the microscopic picture tended to resemble that seen in non-reactive eyes with the exception that plasma cells persisted in the iris and peripheral corneal stroma. The longest interval from intracorneal challenge to sacrifice was 19 days; hence the eventual fate of these cells was not studied.
Discussion

Using a system in which locally sensitized autologous lymph nodes were placed in the anterior chamber, the present study has demonstrated the clinical and pathologic features of a secondary immune response within a previously nonsensitized eye. Since the primary contact with antigen and initial cellular proliferation was performed outside the eye, it may be considered that the subsequent development of an inflammatory response following intracorneal challenge with the same antigen is an example of local adoptive immunity within the eye bearing these pre-stimulated lymph node fragments. Intracorneal challenge of the normal eye with homologous antigen gave no evidence of systemic sensitization. Intracorneal stimulation of the lymphoid tissue was demon-
strably specific for homologous antigen and resulted in severe clinical uveitis accompanied by histologic evidence of a secondary response.

Recent experiments on the histologic appearance and demonstrable antibody formation within lymphoid tissue during the secondary immune response by Thorbecke and colleagues have shown the importance of secondary follicle formation during this stage of immunization. These investigators have shown that antibody production by immature plasma cells outside the proliferating follicles is temporally related with increase in the size of the follicles themselves. In the present experiments, well-developed plasma cells typically seen during the production of antibody were consistently present both at the periphery of the implant and in the surrounding iris stroma following appropriate challenge with homologous antigen. The peripheral corneal stroma was also shown to be infiltrated by plasma cells in examples examined later after a secondary stimulation.

It is apparent, then, that severe anterior uveitis can be produced by the antigenic stimulation of competent cells demonstrating specific immunologic memory. Both the clinical and pathologic features of this reaction resemble those previously described following active primary stimulation of the eye with subsequent antigenic challenge. The larger question of the pathogenesis of this reaction remains. Though specific antigen is requisite to the development of the lesion, it is not clear whether the act of antibody production alone or the subsequent interaction of residual antigen and newly formed antibody is responsible for the severe inflammatory reaction and resultant tissue damage. It was shown in this study that sensitized lymph node fragments remained quiescent in situ with no evidence of clinical uveitis until homologous antigen was introduced. It is probable that small amounts of antibody were formed during this quiescent period, however, and small numbers of plasma cells were present within these transplants. Only following introduction of homologous antigen was the stimulus for such antibody production sufficient to cause uveitis. During this stage it is likely that the presence of specific antigen results in immune complex formation with newly synthesized antibody. Such complexes are known to bind complement and release pharmacologically active agents resulting in vasodilatation and tissue damage.

Further use of this technique might yield useful information on settling this question of pathogenesis. The use of hyperimmune lymphoid fragments and comparison of their behavior to that of relatively quiescent lymphoid tissue would permit at least a preliminary evaluation of the possible pathogenetic effects of ectopic antibody production in the absence of free antigen. The use of this technique for studying immunological reactions in general and more specifically defined ocular immunopathologic events has a number of advantages. Clinical examination of the eye is a familiar procedure for most workers in ophthalmology and a readily acquired technique for others interested in more general immunologic phenomena. Observations are easily made through a relatively clear cornea and evolution of the response is apparent. The anterior chamber of the eye thus provides both a transparent chamber for examination of immunologically competent tissue as well as a sensitive indicator of immunopathologic events.

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