Local adoptive immunity in the eye
II. Ocular response following lymph node allograft and specific antigenic challenge

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The rabbit anterior chamber was used for implantation of specifically sensitized allogeneic lymph node tissue. Initial vascularization was followed by a primary uveitis corresponding to a local graft-versus-host reaction (GVHR) which resolved after 4 to 6 days. Intracorneal injection of specific antigen in quiescent eyes bearing viable lymph node allografts was followed by an intense inflammatory reaction characterized by iris hyperemia, flare and cells in the anterior chamber, and varying degrees of corneal clouding and occasionally Wessely ring formation. Microscopic examination of reactive eyes showed evidence of secondary reactive follicle formation in the transplant, increased number of plasma cells both within the graft and surrounding iris stroma, and an early polymorphonuclear response in the anterior chamber and cornea. While increased levels of precipitating antibody could be demonstrated from aqueous of reacting eyes, recipient animals were uniformly negative in response to skin test with specific antigen, and no serum antibody could be demonstrated by Ouchterlony agar diffusion. No inflammatory response resulted following nonspecific antigenic challenge or specific challenge after rejection of the graft. Prior irradiation (1,000 r.) of allograft recipients depressed both the GVHR and the specific antigen-dependent inflammatory reaction. The results indicate that adoptive immunity can be transferred to the eye of a systemically nonreactive host by viable immunologically competent allogeneic lymphoid cells prior to their rejection, and that significant anterior uveitis results from the interaction of antigen and locally produced specific antibody.

Key words: adoptive immunity, allograft, antibody formation, immune complex, uveitis

The pathogenesis of noninfectious ocular inflammation is poorly understood. Experimental models of immunologically specific inflammatory responses leading to ocular injury have been under investigation in a number of laboratories for some time, and the suspicion that tissue damage occurs through interaction of antigenic substances and local defense factors within the eye has been considered to be of prime pathogenetic importance in this location. Silverstein has demonstrated experimentally that the rabbit eye, previously sensitized by the intravitreal injection of soluble antigen, retains immunologic memory for many months and will respond to intravenous administration of
the same antigen with an intense uveal inflammatory response.

Recently it has been found that quite similar inflammatory events may be produced by reaction of specific immunologically committed cells placed in the eye before the administration of antigen in that location. Transplantation of a small fragment of sensitized autologous lymph node tissue into the anterior chamber was effected with minimal trauma, and thereafter eyes bearing such grafts remained quiescent until challenged either intracorneally or systemically with specific antigen to which the lymph node had been originally sensitized. Following such challenge there occurred prompt anterior uveitis and precipitin ring formation in the case of intracorneal antigen administration. Autologous lymph node grafts were shown to increase in cellularity and secondary reaction center formation was present.

The present studies were undertaken to investigate the pathogenesis of this local adoptive immune response, using allografts of presensitized lymphoid tissue. Any degree of systemic immunity inherent in the autograft experiments described above could therefore be eliminated and a clearer estimation made of the contribution of both the local specific immune as well as host nonspecific factors.

Materials and methods

Animals. Albino (New Zealand white) and pigmented (chinchilla) rabbits of both sexes weighing between 3 and 5 kilograms were used for all experiments. The rabbits were housed in separate cages and fed diets of Purina rabbit chow and water ad libitum. For allograft experiments, pigmented or albino rabbits were randomly selected as donors; in all cases albino rabbits served as recipients.

Sensitization and transplantation. Donor rabbits were prepared by the injection of 1 mg. of ovalbumin (Nutritional Biochemicals Corp., Cleveland, Ohio) in complete Freund's adjuvant into the subcutaneous tissues of one or both ears. After one to two weeks the draining preauricular lymph node was removed by aseptic "no-touch" technique, placed in Hank's balanced salt solution, and kept in a Petri dish over ice.

Animals were anesthetized by subcutaneous injection of 25 mg. of promazine hydrochloride and a slow intravenous injection of up to 100 mg. of sodium pentobarbital. The cornea was anesthetized by instillation of 1 or 2 drops of 0.5 per cent proparacaine hydrochloride ophthalmic solution. Fragments of cortical lymph node tissue, 3 mm. in diameter, were introduced into the anterior chamber through a 4 to 5 mm. incision in the cornea just central to the limbal margin. The grafts were then manipulated by gentle stroking of the cornea to a final position 2 to 5 mm. away from the incision site. The corneal wound was closed by 8-0 ophthalmic silk suture on anatraumatic needle. In all instances reconstitution of the anterior chamber, lost through leakage of aqueous at the time of incision, was complete within 30 minutes. Evidence of initial surgical trauma passed by one to two days, as judged by the normal appearance of both the cornea and the iris.

Antigenic challenge. Animals were challenged four to 31 days following transplantation of the lymphoid tissue by injection of 10 μg of either homologous (ovalbumin) or heterologous (bovine gamma globulin) antigen directly into the central cornea. Using a No. 27 hypodermic needle and a 0.25 ml. syringe, approximately 0.01 ml. volume was injected. Fig. 1 shows a typical experiment of this series. In a number of instances eyes bearing sensitized lymphoid tissue grafts were not subsequently challenged but taken for histologic examination at various times after implantation of the lymph node.

Studies of irradiated recipients. Albino rabbits received 1,000 r. total body irradiation in a single dose from a van der Graaf generator calibrated to deliver 25 r. per minute. Transplantation of the lymph node grafts as described above was performed one day after irradiation, and the cornea challenged with homologous or heterologous antigen three to 13 days later.

Study of killed grafts. In another series of animals, similarly sensitized lymph node fragments were subjected to rapid freezing to -70° C., followed immediately by warming to 37° C. The tissue was placed on sterile aluminum foil for such treatment and underwent ten freeze-thaw cycles in less than 15 minutes, following which it was transplanted to the anterior chamber of one eye of the recipient. The opposite eye received intact lymph node tissue from the same donor. Intracorneal injection of specific antigen was performed simultaneously in both eyes eight days following implantation.

Immunologic studies. All animals bearing allografts were skin tested on the day of intracorneal challenge by intracutaneous injection of 10 μg of ovalbumin and bovine gamma globulin (BGG). Serum and aqueous, obtained at the time the animals were killed, were placed in the
Fig. 1. Experimental design used in the major series of animals. Seven days following subcutaneous inoculation of 1 mg. of ovalbumin in complete Freund's adjuvant, the draining preauricular lymph node was removed from the donor and cortical tissue placed in both recipient eyes. Seven days thereafter, intracorneal inoculation of specific antigen was performed on the left eye and heterologous antigen on the right. Evaluation of results and sacrifice of the recipient generally took place 24 to 48 hours following challenge.

center wells of agar diffusion plates. Outer wells were filled with eight doubling dilutions of ovalbumin in saline, starting at 1 mg. per milliliter.

Clinical evaluation and histologic techniques. All animals were observed daily and a Zeiss slit lamp was used to clarify gross observations. Photographs were obtained regularly to record the clinical progress of sequential changes. The degree of clinical reaction was scored on a scale of 0 to 4+, depending on the relative intensity of ocular inflammation, as follows: 1+, moderate iris and limbal hyperemia; 2+, blephorospasm, conjunctivitis, and severe hyperemia of the iris; 3+, edema of iris and aqueous flare with cells; 4+, corneal clouding, Wessely ring formation, or hypopyon. The eyes were removed immediately following death and fixed in alcohol-formalin. Hematoxylin and eosin as well as methyl green—pyronin stains were routinely performed for histologic evaluation of this material. Portions of the lymph node used for transplantation were also similarly processed.

Results

The natural history of lymph node allografts in the anterior chamber. Upon recovery from initial operative trauma, the appearance of allografted eyes differed markedly from autograft controls. Usually beginning about Day 3, an intense iritis with occasional flare developed in all allografted eyes, most marked in the region of the graft itself. Over the succeeding four to five days, this reaction slowly waned, until by Day 7 iris injection was confined to 1 mm. surrounding the graft. Small blood vessels could be easily observed to enter the pinkish-tan lymphoid tissue at this time. During the next week allografts did not change markedly in appearance except for the encroachment of a thin veil of iris about their periphery. Starting at about Day 14, grafts became progressively paler in color until only a small residual white scar was present on the iris face. On the contrary, autografts retained normal color and vascularity through the entire period of observation, up to 150 days.

Histologically all allografts and autografts demonstrated vascularization by the second day following implantation as judged by filling of postcapillary venules within the parenchyma of the lymph node. During the period of iritis noted above, an intense mononuclear reaction was present within the iris stroma at the base of the graft, extending through the full thickness of the iris and laterally several millimeters beyond the graft margin. By Day 7 this mononuclear infiltrate and the accompanying dilatation of capillaries had diminished markedly. Allograft rejection was noted in material removed after Day 15 and consisted of decreased numbers of lymphoid cells within the graft and a concomitant increase in fibrous tissue, until by Day 31 only a small scar remained in the anterior iris stroma. The appearance of the lymph node prior to rejection was similar to that removed from the donor animal at the time of implantation, with the exception that in numerous instances dilated endothelial-lined spaces packed with small
lymphocytes were present within the graft itself. Reticulin stains demonstrated that these spaces were indeed vascular in structure and probably represented pre-existing lymphatic channels lacking the normal communication to efferent lymphatics because of the ectopic location.

Host rabbits receiving 1,000 r. total body irradiation one day prior to implantation of the allograft demonstrated only mild initial uveitis during the week following grafting. Histologically, minimal round cell infiltrate was confined to the immediate graft bed, and this did not persist after the sixth day. In animals so treated, grafts survived essentially intact for the duration of the experiment (15 days) and did not undergo the progressive destruction and replacement by scar tissue noted above. Similarly, freezing and thawing of the lymph node allograft prior to implantation completely abolished the initial uveitis. The contralateral eye grafted with untreated lymph node tissue from the same donor underwent transient iritis during the first week of graft residence.

Response following antigenic challenge. Following intracorneal challenge with homologous antigen, rabbits bearing viable presensitized lymphoid tissue grafts responded in a fairly typical fashion. An inflammatory reaction was noted, usually beginning by six hours and reaching its peak by 48 to 72 hours, after which it slowly resolved. Depending on the intensity, the clinical reaction consisted of vasodilatation of the iris, fixed or slowly reactive pupil, proteinaceous precipitate in the anterior chamber, and corneal clouding with or without precipitin ring formation. Challenge with heterologous antigen in the grafted eye, or with homologous antigen in a nongrafted eye, produced no such inflammatory response. Similarly, eyes bearing frozen-thawed lymph node fragments did not respond.

The earliest histologic changes in the reactive eyes consisted of polymorphonuclear leukocytes within the anterior chamber, especially at the angle, frequently enmeshed in a fibrinous protein precipitate, as demonstrated in Fig. 2. A mixed inflammatory cell population was present within the iris stroma, consisting of polymorphonuclear leukocytes, lymphocytes, and occasional plasma cells. The infiltrate was most intense in the area adjacent to the graft, and as the severity of the reaction increased most of the iris and ciliary body became involved. Fig. 3 shows the character of such a reaction in the iris of an animal challenged 24 hours previously and the contralateral eye challenged with heterologous antigen at the same time. Within the graft itself, secondary follicles became more prominent later after challenge and there was a pronounced increase in plasma cells, many containing Russell bodies within their cytoplasm both within the graft and adjacent iris stroma. In animals receiving 1,000 r. prior to grafting, the clinical severity of the response was reduced in all parameters, though the lymph node graft contained approximately the same number of cells, and following antigenic response a large number of plasma cells and secondary follicle formation were also present (Fig. 4). Corneal swelling was marked in those animals in which uveitis was most severe and precipitin ring formation was noted, as seen in Fig. 5. Even when this feature was not pronounced, numerous polymorphonuclear leukocytes were present within the corneal stroma following specific antigenic challenge. Again, animals receiving total body irradiation showed reduced numbers of polymorphonuclear leukocytes within the

<table>
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<tr>
<th>Days after transplantation</th>
<th>Number of animals</th>
<th>% reacting to specific challenge</th>
<th>Average degree of reactivity*</th>
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<tbody>
<tr>
<td>4-8</td>
<td>16</td>
<td>100</td>
<td>3+</td>
</tr>
<tr>
<td>11-15</td>
<td>5</td>
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<tr>
<td>16-31</td>
<td>7</td>
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*Graded clinically 1+ to 4+ as outlined in Materials and methods section.
Fig. 2. Appearance of the anterior chamber angles in a recipient rabbit 24 hours after specific (A) and nonspecific antigenic challenge (B). Lymph node grafts from adjacent fragments of a single donor node are still intact at this time. Note the fibrinous exudate containing numerous cells, including many disintegrating polymorphonuclear leukocytes, in the eye reacting to ovalbumin (A) compared to the nonreactive right eye (B). (Hematoxylin and eosin. Original magnification x200.)

corneal ring and fewer scattered through the corneal stroma.

Table I summarizes the results of homologous antigenic challenge in 28 animals bearing sensitized lymph node fragments in the anterior chamber. For uveitis to result from specific antigenic challenge, it is obvious that a critical time period existed following lymph node transplantation. All animals challenged between Days 3 and 8 after grafting responded with a severe uveal and corneal inflammatory reaction, with an average degree of reactivity of 3+. Only three animals challenged in the period between Days 11 and 15 showed any degree of ocular reactivity, and after Day 16 no inflammatory reaction was detectable either clinically or histologically.
Little or no graft tissue could be identified in animals killed during this last time period.

**Immunologic studies.** Donor animals supplying lymph nodes were skin test positive for ovalbumin, with erythema and induration usually present before 24 hours and persisting for from 48 to 72 hours. No response to BCG was detected in these animals. Skin tests using ovalbumin and BCG were routinely performed on all recipient animals at the time of intracorneal challenge. In no instance was a positive skin test noted to either antigen.

Ouchterlony double diffusion analysis of the serum of recipients bearing presensitized lymph node tissue was uniformly negative even after intracorneal challenge with homologous antigen. The aqueous from reacting eyes taken at the time of sacrifice demonstrated precipitating anti-
body to ovalbumin in approximately 50 per cent of the animals tested. Aqueous from the contralateral eye tested with BGG showed either trace or no antibody to ovalbumin. Fig. 6 represents results of such experiments in both intact and irradiated recipients. No quantitative difference in the amount of antibody present in the aqueous could be established between these two groups.
Fig. 6. Ouchterlony agar diffusion analysis of aqueous from reacting (OS) and nonreacting (OD) eyes 24 hours after intracorneal challenge. The center wells contain unconcentrated aqueous and the peripheral wells doubling dilutions of ovalbumin starting at 1 mg. per milliliter in well 1. Upper set (A) contains aqueous from a normal recipient and lower set (B) from an irradiated recipient. Note the small amount of antibody in the right eye challenged with nonspecific antigen in the irradiated recipient and the greatly increased production within 24 hours after ovalbumin challenge. Sera from both these animals were negative.

Discussion

This study provides direct evidence of the ability of presensitized immunologically competent cells transplanted to the anterior chamber to produce uveitis following specific antigenic challenge. This contention is supported by: (1) the histologic appearance of the transferred lymph node which demonstrated formation of secondary follicles as well as increased numbers of plasma cells; (2) the ability to recover precipitating antibody from the aqueous of reacting eyes bearing lymph node allografts; and (3) the inability to detect antibody in normal or irradiated hosts in serum by Ouchterlony double diffusion analysis or by cutaneous challenge.

The immunologic specificity of this reaction is demonstrated by the inability of heterologous antigen to cause uveitis in eyes bearing sensitized lymph node fragments. It was also found that implantation of lymph node fragments frozen and thawed through multiple cycles did not prepare normal hosts for a uveal reaction upon specific intracorneal challenge. This negative finding also argues against the possibility of chance carryover of antigen within the lymph node to the normal host, resulting in local sensitization and subsequent reactivity.

The adoptive nature of this response is also supported by the time course of the uveal and corneal reaction which developed rapidly following specific antigenic challenge. Within 24 hours after intracorneal injection, corneal clouding and in many cases precipitin ring formation could be demonstrated. Histologically, both the lymph node graft and adjacent iris stroma as well as ciliary body contained numerous plasma cells. An important point in favor of the adoptive nature of this response is shown by the fact that only prior to lymph node graft rejection could the inflammatory sequence of events be elicited. If host cells had received either antigenic stimulus or had been recruited by specific immunological "information" from transferred cells, sensitivity to ovalbumin should have persisted beyond the time of graft rejection. Such persistence of immunologic memory could not be demonstrated.

The manifest ability to produce antibody upon specific challenge during the period of viability of the lymph node fragment was retained even after the exposure of the host to 1,000 r. irradiation prior to grafting. It was also clear in this series of animals, however, that such treatment reduced the degree of clinical reactivity resulting from specific antigenic challenge. The clinical response following antigen
administration is not, therefore, solely due to interaction of antigen with either preformed or newly synthesized antibody but is to a considerable degree dependent upon a contribution of the host. That this phase of the response is at least partially sensitive to the dose of irradiation used in these experiments is reflected in the relative paucity of inflammatory cells within the iris and corneal stroma in the irradiated animals compared to their nonirradiated control group.

The initial clinical and histologic evidence of iritis occurring in all lymph node-allografted animals prior to intracorneal challenge which disappeared by the sixth day following transplantation strongly suggests a graft-versus-host reaction. This interpretation gains support from the finding that only living allogeneic immunologically competent cells call forth this initial response. Lymph node grafts of autologous origin or allografts of immunologically non-competent tissues, including skin and thyroid, fail to evoke an inflammatory reaction. This initial uveitis waned after Days 4 to 6 and did not interfere with the interpretation of subsequent inflammatory responses due to intracorneal injection of specific antigen. Freezing and thawing the lymph node allograft prior to transplantation completely eliminated the initial primary response. This further suggests that living immunologically competent cells are required for production of the graft-versus-host reaction. Additional support for the initial response being graft-versus-host in character is gained by the results seen in the irradiated animals. Following superlethal levels of irradiation, the initial inflammatory response was considerably diminished, though animals so treated were able to respond to intracorneal injection of specific antigen. Elkins' reported that the local graft-versus-host reaction requires the presence of radiosensitive host cell population to interact with competent graft cells in order to permit the lesion to attain maximum size. In our studies, irradiated hosts developed a meager initial infiltrate following transplantation of lymph node, whereas normal animals had extensive mononuclear cell inflammatory reaction adjacent to the graft and within the surrounding iris stroma. We interpret this finding as similar to those of Elkins in which whole body irradiation to F-1 hybrids subsequently given parental lymphoid cells markedly diminished the extent of the graft-versus-host reaction.

The model system used in these studies serves to demonstrate that severe inflammatory reaction may occur within the uveal tract and corneal tissues as a result of antigenic stimulation of immunologically competent memory cells. The clinical and pathologic features of this reaction resemble those previously described following primary sensitization of the eye and subsequent intravenous challenge. Double diffusion analysis using aqueous from quiescent eyes bearing sensitized but unchallenged or heterologous antigen-challenged lymph node grafts occasionally demonstrated the presence of low levels of specific antiovalbumin antibody. Likewise, histologic examination of such tissue shows the presence of a considerable number of plasma cells within the unstimulated node. The simple presence of cells engaged in the process of antibody formation within the graft does not result in clinically or histologically demonstrable ocular inflammation. We feel the data favor the following interpretation: The initial step following intracorneal challenge consists of reaction to locally formed antibody of graft origin and recognition by viable lymph node cells of specific antigen to which the graft had initially been sensitized. This leads to an anamnestic response consisting of cellular proliferation with plasma cell differentiation and increased levels of specific antibody production by the allograft. Residual antigen then interacts locally with antibody, leading to tissue damage. The role of the host in the development of the inflammatory response is to supply nonspecific factors.
after the immunologic event of recognition and antibody production. These would include the various components of the complement system including the generation of chemotactic factor from C5 and the majority of inflammatory cells infiltrating the uveal tract, anterior chamber, and corneal tissues. The precise role of complement in generation of chemotactic factors responsible for recruitment of these non-specific inflammatory cells is currently under investigation.

One final point deserves consideration. The anterior chamber of the eye has long been considered a privileged site for survival of allografts. The present study does not support this view, since allografts of lymphoid tissue were seen to undergo primary rejection eight to 15 days after transplantation to normal hosts. As in the cases of primary grafts of skin and thyroid, the ultimate rejection of lymph node allografts in the anterior chamber was readily demonstrable. In addition to histologic evidence of rejection, the added parameter of function in terms of the ability to mount a secondary immune response was specifically abrogated by the rejection phenomenon.

REFERENCES