**LETTERS**

**Normal Rat Retina Lacks Significant Numbers of ED2-Positive Macrophages**

We want to correct some statements that we published in an earlier volume of *IOVS*.\(^1\) We described microglia and macrophages in the normal rat retina and the response of these cells during the endotoxin-induced uveitis model (EIU). Retinal wholemounts from normal rats and at various time points after endotoxin injection were stained with the monoclonal antibodies ED1 and ED2. We reported the staining pattern and densities (196–271 cells/mm\(^2\)) of these two antibodies to be essentially similar and described a massive influx of macrophages 24 to 96 hours after endotoxin injection. We reported that these cells were both ED1\(^+\) and ED2\(^+\). Since then we have repeated the experiments and performed similar protocols in collaboration with Dr. Paul McMenamin, who has extensive experience with these monoclonal antibodies, during a sabbatical visit he paid to our Institute. We experienced some difficulties in the staining of ED2, which caused us to review the material originally published in *IOVS*. With the aid of Dr. McMenamin, we performed extensive tests with the monoclonal antibody batches used in the original experiments, compared them with new batches, and concluded that retinal microglia, i.e., the macrophages within the retina, are weakly ED1\(^+\) but ED2\(^−\). The original findings that these cells were ED2\(^+\) was in fact erroneous and appeared to be due to an error in labeling of antibodies at the time of aliquoting. It is important that this issue be clarified, because a number of studies of normal rat retinal tissue have failed to find significant numbers of ED2\(^+\) macrophages in the retina.\(^2,3\) Furthermore, no influx of ED2\(^+\) macrophages was noted in EIU by Pouvreau et al.\(^2\) We apologize for any inconvenience caused to our colleagues as a result of this error.

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**References**


**Limitations in the Study of Immune Privilege in the Subretinal Space of the Rodent**

The immune privilege of the subretinal space has been a subject of recent interest\(^1,2\) because of the potential of allogeneic retinal transplantation in the treatment of several diseases for which we currently have inadequate therapies, such as hereditary retinal degeneration (e.g., retinitis pigmentosa) and age-related macular degeneration. The well-defined immunogenetics of the rodent make it an attractive species in which to conduct such experiments, particularly because some rodent models of hereditary retinal degeneration resemble human disease and have been well characterized (e.g., the *rd* mouse and Royal College of Surgeons rat). However, the small size of the rodent eye and the large size of the rodent lens severely compromise the ability of that species to provide a definitive answer to this problem.

First, the large lens and small vitreous cavity (<5 μl) of the rodent eye require a transscleral (or more anatomically disruptive) approach to deposition of tissue or antigen in the subretinal space. A transvitreal approach is not anatomically possible. Disruption of the integrity of the retinal pigment epithelium (RPE) must occur with a transscleral approach to the subretinal space. Thus, an important anatomic barrier for the establishment of immunologic privilege in the subretinal space will be breached, with the possible compromise of the immunologic properties of this site. Second, the deposition of tissue or antigen in the subretinal space using a transscleral approach does not allow the investigator to control carefully the volume of inoculum placed in the space—much of it invariably leaks out of the scleral entrance site—or to place the inoculum precisely in the subretinal space and not in surrounding compartments.

In a review of the light micrographs in the article by Zhang and Bok,\(^1\) allogeneic RPE cells appear to be in the subretinal space as well as in the choroid. The presence of allogeneic cells in the latter would severely compromise a study of the immunologic privilege of the subretinal space.

We have used several different surgical modifications of the transscleral approach in the rodent, with many different instruments and needles, and have not been satisfied that we can control either the quantity or the placement of the inoculum satisfactorily. Figure 1 depicts a rat eye after the injection of 3 μl India ink into the subretinal space. The ink is delivered through a 30-gauge blunt cannula attached to a Hamilton syringe, through a small radial sclerotomy anterior to the equator made with a myringotomy blade. Although part of the inoculum is in the subretinal space (Fig 1A), it can also be seen in the choroid and vitreous cavity on adjacent histologic sections (Figs. 1B, 1C, respectively).

We believe it is critical for any study of immune privilege in the subretinal space to demonstrate convincingly the ability to place a precise quantity of the inoculum in that space, without contamination of the adjacent compartments. Even with such demonstrated expertise, the conclusions reached must be qualified by the anatomic breach of the RPE barrier. It is for these reasons, as well as others, that we believe an
The Authors Respond

Al-Amro et al. have questioned the use of rodents as experimental subjects for the study of immune privilege in the subretinal space. They accurately state that the eyes of mice and rats are not suitable anatomically for transvitreal placement of subretinal tissue grafts and conclude that a transscleral-choroidal approach is the only reasonable option. This approach, they claim, compromises the blood–retinal barrier and is prone to deposition of allogeneic cells in the choroid. Our Figure 1 is cited as an example of this problem. They assert that this would “severely compromise” studies of immune privilege in the subretinal space, and they propose a transvitreal approach in larger animals as a solution to this problem.

We have reviewed the tissue sections illustrated in Figure 1 of the referenced work and find no evidence of allogeneic cells in the choroid. Al-Amro et al. are apparently confusing erythrocytes in the choroidal vessels with pigmented allogeneic cells. We do not deny that allogeneic cells could be deposited in the choroid with a transscleral approach, but our Figure 1 is not an example that should be used to illustrate this point. Nonetheless, even if allogeneic cells are deposited in the choroid, the point is probably moot. We believe that compromise of the outer blood–retinal barrier by a small wound of the type used in our studies is unlikely in the normal rodent retina. A wound of this type would heal quickly, just as quickly as a wound that would breach the inner blood–retinal barrier through the transvitreal approach suggested by Al-Amro et al.

Thus, we do not agree that the advantage offered by the well-defined immunogenetics and availability of congenic strains should be sacrificed a priori in the interest of the issues perceived by Al-Amro et al. Until the immunologic consequences of a transvitreal versus a transscleral approach are compared experimentally, the issues are conjectural.

The central purpose of our study of retinal pigment epithelial graft behavior in the Royal College of Surgeons (RCS) rat was to examine the question of the immune response in the subretinal space under conditions in which the immunogenetics of the donor and recipient were defined. Previous studies involving retinal pigment epithelium grafting in RCS rats were performed with outbred, donor Long–Evans rats for which the immunogenetics are undefined. Our conclusion was that there is a slow rejection of retinal pigment epithelium grafted into the subretinal space of RCS rats when donor class I or class II antigens differ from those of the host. A study of this kind would not be interpretable with outbred animals, and there are currently no inbred, immunogenetically defined species with larger eyes.
References


Al-Amro et al. question the validity of studies in which immune privilege is examined in the subretinal space of the rodent. They base their concern on the size difference between rodent eyes and eyes of larger mammals and the obvious differences this makes for surgical maneuvers that are used to gain access to the subretinal space. They suggest that meaningful translation of information gained experimentally to allogeneic retinal transplantation in man requires that experiments to examine this issue be performed in eyes of larger species.

The argument of Al-Amro et al. is based, at least in part, on an experiment in which India ink placed in the subretinal space of a rat eye was found in the subretinal space, the choroid, and vitreous cavity. They argue that escape of the inoculated ink outside the subretinal space “severely compromises a study of the immunologic privilege of the subretinal space.” Perhaps they are correct. But if they are, then immune studies after antigen injection into the anterior chamber of rodents eye suffer the same compromise. Injections of P815 tumor cells into the BALB/c AC result in tumor cells seeded along the needle tract. Consequently, tumor cells infiltrate the corneal stroma, sclera, and posterior uvea. Yet, recipient mice display anterior chamber-associated immune deviation (ACAID), and P815 cells enjoy unlimited immune privilege in their eyes. These considerations lead me to recommend circumstance when reaching conclusions that require “absolutes.” There are very few absolutes in experimental science, and injections in the subretinal space of large animals are no more “absolutely” delivered into the target space than are injections into the subretinal space of rodents.

What saves ACAID experiments from being unreliable is the type of controls that are performed. Control injections of antigen into the cornea, sclera, or subconjunctiva do not elicit ACAID, whereas injections of antigen into the anterior chamber do. More to the point, application of herpes simplex virus (HSV)-1 onto the scarified corneal surface induces conventional delayed hypersensitivity, whereas injection of the same antigens into the anterior chamber induces ACAID. Simultaneous injection of HSV-1 into the anterior chamber and application of HSV-1 to the scarified cornea results in ACAID. Thus, at least for immune deviation, placement of antigen into an immune-privileged site has a dominant effect when the immune system is confronted by antigen from a nonprivileged site. I suspect the same thing happens when antigenic material is placed in the subretinal space, although no current evidence exists to support this suspicion.

I am reminded of a wonderful book, *The Dancing Wu Li Masters,* in which G. Zukav points out that every time we do an experiment, we change the system. Any experimental results we obtain are derived from altered, rather than intact, biologic entities. Even transvitreal approaches to the subretinal space of eyes of large animals necessarily “change the system.” So the issue is not whether one approach does, and the other approach does not, change the system, it is “how can we change the system as little as possible, and still obtain important information.” I believe that the benefits that accrue from rodent studies (well-defined immunogenetics, many congenic strains, diverse biologic reagents) outweigh the concessions that must be made to reduced size and accessibility. Although important experiments can be performed on eyes of larger animals, those experiments are unlikely to yield information that is important immunologically. The only animals with large eyes for whom reagents are available in sufficient diversity and array are human beings. Thus, rodents are the only reasonable alternative to answer immunologic questions about immune privilege in the subretinal space.

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References