Immunobiology of Langerhans Cells on the Ocular Surface

I. Langerhans Cells Within the Central Cornea Interfere With Induction of Anterior Chamber Associated Immune Deviation

Jo S. P. Williamson,* Salvador DiMarco,† and J. Wayne Streilein*

Inoculation of P815 tumor cells into the anterior chamber of eyes of BALB/c mice produces Anterior Chamber Associated Immune Deviation (ACAID) whereby delayed hypersensitivity responses to the minor II antigens of the tumor cells are suppressed. In our laboratory, impaired delayed hypersensitivity is obtained in the majority, but not all normal BALB/c recipients. In the minority, typical delayed hypersensitivity responses, similar to those induced by subcutaneous immunization with tumor cells, are observed. Retrospective examination of eyes of animals that develop delayed hypersensitivity following intracameral tumor cell injection revealed a high incidence of a corneal disorder in which significant numbers of Langerhans cells are found within the corneal epithelium. BALB/c mice with corneal lesions characterized by opacity and calcium deposition, or whose corneas had become infiltrated with Langerhans cells induced to emigrate by a superficial cautery wound, received intracameral injections of P815 cells. Minimal or no significant suppression of delayed hypersensitivity was obtained, suggesting that ACAID induction had not taken place. These results suggest that the presence of Langerhans cells in the central corneal epithelium mitigates against ACAID induction. Invest Ophthalmol Vis Sci 28:1527-1532, 1987

Exogenous antigens placed in the anterior chamber of the eye induce an unusual spectrum of immune responses, termed anterior chamber associated immune deviation ACAID.1,2 Critical factors within the anterior segment which are thought to play a role in ACAID induction are (1) injected antigens are released directly into the blood vasculature via the trabecular meshwork-Canal of Schlemm, rather than passing first via lymphatics to a draining regional lymph node; and (2) the lining of the anterior chamber—iris, corneal endothelium, and meshwork—is devoid of cells constitutively expressing class II major histocompatibility determinants and unlikely to provide accessory cell function.3,4 In the case of the former, (1), it is believed that direct intravenous release of antigen permits the initial stages of processing and presentation to take place in the spleen, an organ in which down-regulation of certain types of immune reactivity may be dominant.5 The fact that splenectomy prior to anterior chamber inoculation of antigen prevents ACAID supports this argument.1,6 In the case of the latter, (2), absence of class II -expressing cells within the anterior chamber insures that intracameral processing and presentation of antigen does not take place. The observation that normal alloantigenic corneas (ie without Langerhans cells in the central region) grafted heterotopically to the body wall prime for cytotoxic T cells, but not delayed hypersensitivity is relevant to this argument.7 Peeler and Niederkorn have demonstrated that allogeneic corneas containing experimentally-induced central Langerhans cells when placed heterotopically induce both delayed hypersensitivity and primed cytotoxic T cells. Perhaps Ia+ antigen-presenting cells contaminating the cornea which lines the anterior chamber can mitigate against ACAID induction.

We have recently become aware that a significant proportion of putatively normal BALB/c mice in our colony develop a corneal lesion, which is characterized by unilateral or bilateral, central or slightly nasal corneal opacities, frequently accompanied by localized anterior stromal neovascular growth—from the limbal vasculature towards the lesion. Histopathologic findings revealed minimal inflammatory cell infiltration and lesions were confined to the corneal...
epithelium with mineralization (calcium deposition) of the subepithelial zone, similar to the corneal opacities described recently by Van Winkle and Balk. The epithelium of corneas bearing such lesions contained Langerhans cells at the limbus, as has been observed with normal corneal epithelium, but in addition, a significant number of Langerhans cells were also found unevenly distributed in central corneal epithelium. We have also realized recently that a significant minority of BALB/c mice inoculated intracamerally with P815 tumor cells fail to develop ACAID. It occurred to us that the two phenomena may be related. In this communication we describe the results of ACAID induction in normal and lesion-bearing eyes. We have found that Langerhans cells contaminating the corneal epithelium may prejudice the anterior chamber against promoting deviant immune responses to intracameral inoculated antigens.

Materials and Methods

Experimental Animals

Female BALB/c mice were bred and maintained in our domestic colony. Animals were used between 3 and 5 months of age, and were treated according to the ARVO Resolution on the Use of Animals in Research.

Tumor Cells

P815 mastocytoma cells (DBA/2 origin) were grown in Corning 75 cm² tissue culture flasks (Corning Glass Works, Corning, NY) using RPMI 1640 (GIBCO Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (FCS, Hyclone Laboratories, Logan, UT), Gentamycin (0.02 mg/ml, Boehringer Mannheim Biochemicals, Indianapolis, IN), L-glutamine (2 mM, GIBCO) and Heps (5 mM, GIBCO). Tumor cells were harvested and washed in Hanks balanced salt solution (HBSS, GIBCO) and resuspended in HBSS for subcutaneous (SC) and intracameral (IC) injections.

Injection of Tumor Cells

A modified quantitative technique for depositing a specific number of tumor cells into the anterior chamber of the mouse eye has been described elsewhere. Mice were deeply anesthetized with sodium pentobarbital (0.6 mg/10 g, Barber Veterinary Supply Co., Richmond, VA) given intraperitoneally. P815 cells (10⁵/2 μl) were injected into panels (five to ten animals) of mice. Injected eyes with progressively growing tumors were enucleated on day 7 post inoculation. Positive control mice were immunized with 10⁵ tumor cells subcutaneously into two sites under dorsal skin.

Thermal Cautery Wounds of Corneal Epithelium

Mice were deeply anesthetized during application of a surgical cautery (Bircher Hyfrecator, Los Angeles, CA) to produce a superficial corneal lesion in the right eye. A single application (10 joules/second) was performed with the fine tip of the cautery so as to produce a central, superficial lesion that involved the entire epithelium, and the upper third of the stroma. The surface area of the epithelial lesion was approximately equal to the size of the pupil under normal illumination. In no instance was corneal perforation, cataract formation or vascularization observed following the injury. Topical fluorescein and cobalt-blue light revealed complete re-epithelialization of the wound by 72 hr.

Assay For Delayed Hypersensitivity (DH)

DH was measured as the amount of ear swelling following a SC challenge with P815 cells. Panels of mice that had been primed ten days previously with IC or SC injections were ear challenged with 5 X 10⁵ X-irradiated P815 cells (10,000 R). Irradiated tumor cells were resuspended in HBSS (5 X 10⁷ ml) and 10 μl were injected into the subcutaneous tissue of the right ear. As a negative control, the left ear was uninjected, or injected with 10 μl of HBSS. Both ears of each mouse were measured with a low pressure engineer's micrometer (Mitutoyu 227-101, MTI Corporation, Paramus, NJ) immediately prior to ear challenge, and again 24 and 48 hr later. The amount of ear swelling was calculated as follows:

Specific Ear Swelling = (24 hr measurement — 0 hr measurement of right ear) — (24 hr measurement — 0 hr measurement of left ear) X 10⁻³ mm.

Statistical Analysis

Standard errors of the mean were calculated and the significance of the data was analyzed by student t-test.

Results

Within the recent past, several experiments involving ACAID induction to DBA-2 antigens in BALB/c mice using intracameral inoculations of P815 tumor cells have shown that these animals display a marked reduction of delayed hypersensitivity responses to an intradermal challenge with irradiated tumor cells compared to subcutaneously immunized animals.
The results of our seven most recent experiments summarized in Figure 1, however, show that while the mean of the IC-injected group is significantly less than that of the SC-injected positive controls, it is apparent that there are an uncomfortably large number of IC-injected animals (approximately 50%) that developed ear swelling responses within the positive control range. Close clinical examination of one group of these experimental mice by one of us (JW) led us to suspect that the presence of a corneal disorder might be implicated in the induction of vigorous DH responses of certain mice receiving P815 IC.

**ACAID Induction in Mice With and Without Corneal Opacities**

Groups of age- and sex-matched mice were selected on the basis of careful clinical examination, with the aid of a dissecting microscope, as (1) without corneal opacities or (2) with corneal opacities in the right eye. Each animal received 10⁵ P815 cells intracamerally O.D. Their ears were challenged 10 days later with irradiated P815 cells and the swelling responses measured 24 and 48 hr later. As the results in Figure 2 depict, normal mice developed mean ear swelling responses indistinguishable from negative controls, although one animal did express significant ear swelling. By contrast, animals with corneal lesions developed ear swelling responses significantly higher than both negative controls and the normal mice. However, these intermediate level responses were not of the same magnitude as those displayed by the positive control mice.

In a subsequent experiment, similar panels of lesion-bearing and normal mice were tested for ACAID induction along with a third panel comprised of lesion-bearing mice with stromal neovascularization. As the results in Figure 3 depict, superimposition of neovascularization upon the corneal abnormality did not further enhance the subsequent DH responses. Both types of abnormal mice displayed DH responses to DBA/2 antigens that were significantly positive and intermediate in intensity between negative and positive controls. As before, the normal mice developed ACAID.

**ACAID Induction Following Cauterization of Normal Corneas**

The observation that mice with corneal lesions are significantly less able to develop ACAID in response to intracamerical inoculation of P815 cells is a tanta-
Fig. 3. Delayed hypersensitivity responses of mice following inoculation of P815 tumor cells into anterior chambers of normal eyes (IC), eyes with corneal opacities due to calcium deposition complicated by stromal vascularization (IC/CaD-V), and eyes with corneal opacities without vascularization (IC/CaD). Horizontal lines represent mean values. Mean SC is significantly greater than mean unprimed \( (P < 0.005) \) and mean IC \( (P < 0.02) \); mean IC/CaD and mean IC/CaD-V are significantly different from mean unprimed \( (P < 0.05) \). There are no significant differences between mean SC and mean IC/CaD, or mean IC/CaD-V; nor is there a significant difference between mean IC and mean unprimed.

Fig. 4. Delayed hypersensitivity responses of mice following inoculation of P815 tumor cells into anterior chambers of eyes that received a central corneal cautery wound 14 days previously (IC/CAUT), or normal eyes. Horizontal lines represent mean values. Mean SC is significantly greater than mean unprimed \( (P < 0.005) \) and mean IC \( (P < 0.02) \); mean IC/CAUT is significantly greater than mean unprimed \( (P < 0.005) \). There is no significant difference between mean SC and mean IC/CAUT, nor between mean IC and mean unprimed.

Discussion

While all of the relevant factors responsible for the phenomenon of ACAID have yet to be identified, two features are thought to be critical: (1) antigens placed in the anterior chamber escape directly into the systemic circulation via the trabecular meshwork and Canal of Schlemm; no draining lymphatics nor regional lymph node intervenes before the antigen is processed by the spleen; and (2) the structures lining the anterior chamber (iris, lens, corneal endothelium, trabecular meshwork) are devoid of specialized cells constitutively expressing class II major histocompatibility antigens. It is pertinent that, with regard to (1), splenectomy prior to intracameral inoculation of antigen prevents ACAID,\(^6\) and, with regard to (2), heterotopic grafts of allogeneic corneas devoid of class II bearing Langerhans cells can induce cytotoxic cells without inducing delayed hypersensitivity, whereas grafts containing Langerhans cells can induce delayed hypersensitivity also.\(^7\) Thus, it can be hypothesized that the absence of specialized antigen presenting
cells within the anterior chamber and its lining allows unprocessed antigen to leave via the blood vasculature, travel directly to the spleen, and initiate a unique blend of lymphoid cellular interactions that produce specific suppressors of delayed hypersensitivity.

A simple interpretation of the results reported in this communication would be that Langerhans cells in the central corneal epithelium provide a source of extremely effective, class II MHC-bearing, antigen-presenting cells to the anterior chamber. As a consequence, the minor histocompatibility antigens with which P815 cells confront BALB/c mice are processed by corneal Langerhans cells and presented in highly immunogenic fashion to host T lymphocytes which engender typical delayed hypersensitivity. However, this simple construction belies the complexities which must yet be explained.

If corneal Langerhans cells process P815 antigens following anterior chamber inoculation of the tumor cells, how do the antigens gain access to the Langerhans cells? One possibility is suggested by recent histopathologic examinations of BALB/c eyes following IC P815 injections. Tumor cells are inadvertently deposited along the needle track through the lateral aspect of the cornea; within a short period of time, advancing strands of tumor cells can be observed to grow and migrate extensively between the lamellae of the cornea. Perhaps in this manner antigenic fragments are released near Bowman’s membrane and (1) diffuse into the epithelial compartment where Langerhans cells reside, or (2) are picked up by Langerhans cells that “drop down” from the epithelium through Bowman’s membrane into the superficial stroma. Alternatively, since tumor cells can be found to be invading the corneal epithelium itself, Langerhans cells within that compartment could pick up relevant antigens directly.

If corneal Langerhans cells process P815 antigens in an immunogenic fashion, by what route do they deliver this potent signal to the systemic immune system such that unsuppressed delayed hypersensitivity is achieved? Two possibilities suggest themselves. In the first, it is conceivable that corneas containing Langerhans cells (either as a consequence of lesions of unknown etiology or after cauterization) acquire a lymphatic drainage; if so, then cells bearing P815 antigens would have access by flowing via the lymph to the draining cervical lymph nodes. Since we have already documented that P815 cells are readily detectable in the cervical nodes of mice bearing intracorneal P815 tumors,14 we know this pathway is available. An alternative mode of delivery of the immunogenic P815 signal would be for antigen-laden Langerhans cells to migrate directly into the anterior chamber and exit via the trabecular meshwork and canal of Schlemm. Sullivan et al12 have recently shown that very small numbers of hapten-derivatized Langerhans cells induce vigorous contact hypersensitivity when injected by the intravenous route.

But a simple interpretation of these results does not satisfy for a very good reason. While in the normal eye the central cornea is essentially devoid of Langerhans cells, large numbers of these cells are present normally in the limbus and conjunctiva. It would seem highly probable that the track of tumor cells left by the needle within the stroma after an anterior chamber inoculum would just as easily contact limbal Langerhans cells as central corneal Langerhans cells. Yet, when the cornea lacks Langerhans cells, ACAID is induced; when they are present, ACAID fails to develop. The limbal Langerhans cells seem irrelevant to the process, although the reasons are far from clear. We must also be prepared to consider an alternative possibility: while under normal circumstances very few class II MHC-bearing cells occupy the iris, and none can be found in the trabecular meshwork,3 the possibility exists that the corneal lesion, and/or a central cautery wound to the cornea may be accompanied by infiltration of Ia+ cells into the tissues that line the anterior chamber—iris, trabecular meshwork, etc. While this seems an unlikely explanation for the failed ACAID in our animals, experiments to examine this possibility are currently underway.

Our findings that corneal Langerhans cells interfere with ACAID induction force us to consider all layers of the cornea as relevant to the qualities of the anterior chamber that confer immune privilege upon it. We have proposed that immune privilege in the anterior chamber (and ACAID which we believe to be the cellular basis for privilege) provides the eye with those forms of immunity (antibody, cytotoxic T cells) that provide protection without the risks of architectural disruption that delayed hypersensitivity requires. If that is true, then abnormal corneas that contain Langerhans cells may eliminate the capacity to suppress delayed hypersensitivity responses to invading ocular pathogens. Destructive anterior segment inflammation as well as uveitis may be among the pathologic consequences of this unfortunate circumstance.

Key words: Langerhans cells, corneal dystrophy, anterior chamber associated immune deviation, ocular tumors, delayed type hypersensitivity

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References