Brief Definitive Report

SYSTEMIC IMMUNE UNRESPONSIVENESS INDUCED IN ADULT MICE BY ANTERIOR CHAMBER PRESENTATION OF MINOR HISTOCOMPATIBILITY ANTIGENS

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When carefully controlled numbers of alloantigenic cells are injected into the anterior chamber of the eye, they elicit an unconventional host immune response. This phenomenon, first described in a rat alloimmune system (1, 2), has been successfully adapted to mice. BALB/c mice, injected intracamerally with P815 tumor cells derived from the DBA/2 mouse strain, can be rendered specifically unresponsive to the minor histocompatibility antigens of the DBA/2 strain. These animals accept orthotopic DBA/2 skin grafts for prolonged intervals and allow P815 cells to form tumors at subcutaneous inoculation sites. These findings confirm and extend evidence in favor of the hypothesis that alloantigenic presentation through the anterior chamber of the eye (and perhaps other immunologically privileged sites as well) induces an active immune response that is protective rather than destructive.

The P815 tumor cell line originated in the DBA/2 mouse strain. Cells of this line express surface histocompatibility antigens as well as tumor-specific transplantation antigens (3). P815 has been used extensively to study alloimmune reactions in vitro, especially cytolytic reactions. The DBA/2 and BALB/c mouse strains are related to each other in that both share the same *H-2* chromosomal segment; however, they differ at multiple minor histocompatibility loci (4). Their minor alloantigenic disparities were used to advantage in this study.

Materials and Methods

Experimental Animals. Adult female BALB/c ($H\text{-}2^d$) and DBA/2 ($H\text{-}2^d$) mice were purchased from The Jackson Laboratory, Bar Harbor, Maine and used as experimental subjects when they were between 3 and 5 mo of age.

Tumor Cells. P815 mastocytoma (DBA/2) cells were cultivated in suspensions cultures in Falcon 75-cm^2 tissue culture flasks (Falcon Labware Div., Becton, Dickinson & Co., Oxnard, Calif.) using Dulbecco's modified Eagle's minimal essential medium supplemented with 10% heat-inactivated fetal calf serum and gentamycin (0.05 mg/ml; Schering Corp., Kenilworth, N. J.). EL-4 lymphoma (C57BL/6) was maintained by serial passage as ascites in C57BL/6 mice. Monocellular suspensions of P815 cells and EL-4 cells were washed in Hanks' balanced salt solution (HBSS) and resuspended in HBSS for subcutaneous and intracameral inoculations.

Anterior Chamber Inoculations. A modified quantitative technique for depositing a definite number of tumor cells into the anterior chamber of the mouse eye was employed (5). Mice were deeply anesthetized with 0.66 mg of ketamine hydrochloride (Vetalar; Parke, Davis & Co., Detroit, Mich.) given intramuscularly. The eye was viewed under the low power (8 ×) of a dissecting microscope and a sterile 30-gauge needle was used to puncture the cornea at the corneoscleral junction, parallel and anterior to the iris. The aqueous humor was expressed by

compressing the cornea with the back of a scalpel blade and the evacuated fluid blotted with sterile gauze pads. A micro-glass pipette (~80 μm in diameter) was fitted into sterile infant feeding tube (5 French; Cutter Laboratories, Inc., Berkeley, Calif.) that was mounted onto a sterile 0.1-ml Hamilton syringe (Hamilton Co., Inc., Whittier, Calif.). A Hamilton automatic dispensing apparatus was fitted onto the loaded syringe and was used to dispense 5-μl quantities of P815 cell suspensions. The pipette loaded with P815 cell suspension (2×10^7 cells/ml = 1×10^5 cells/5 μl) was introduced through the puncture site of the cornea and 5 μl of the P815 cell suspension delivered into the anterior chamber. As the needle was withdrawn, the iris prolapsed and plugged the perforated cornea, thereby minimizing leakage of the inoculum.

Subcutaneous Inoculations. 1×10^5 tumor cells (P815 or EL-4) suspended in 0.1 ml of HBSS were inoculated subcutaneously into the right rear flanks.

Skin Grafting. Full-thickness skin grafts were prepared as described elsewhere (6). Grafts were applied orthotopically and wrapped in plaster of Paris bandages. Casts were removed 7 d later, and the grafts inspected for evidence of rejection. Destruction was judged complete when all remnants of surface epidermis were gone. Median survival times were calculated.

Results

P815 tumor cells, in a constant dose of 10^5/inoculum, were injected into the anterior chamber of eyes of panels of DBA/2 and BALB/c mice. A typical growth pattern was observed that was identical for members of both strains: white clumps of tumor appeared in the anterior chamber within 4 to 5 d. Over the next 10 d, the chamber gradually filled with tumor which then grew anteriorly in wedge-shaped manner into the substance of the cornea and finally perforated that structure. These orbital tumors continued to grow for as long as 3 wk, although necrosis frequently intervened, destroying the inoculated eye.

Comparable numbers of P815 cells were then injected subcutaneously into the flanks of similar panels of DBA/2 and BALB/c mice. Progressively growing tumors appeared at inoculation sites in DBA/2 recipients, but no evidence of tumors was found in the flanks of BALB/c recipients (Table I). This set of findings clearly indicates that the anterior chamber of the mouse eye is an immunologically privileged site that allows a histoincompatible tumor cell line injected therein to grow unimpeded.

Next, a panel of BALB/c mice was grafted orthotopically with DBA/2 skin. All grafts were rejected in typical first-set fashion, with a median survival time of 10.8 d (Table II). 2 wk after engraftment, these animals received intracameral injections of 10^5 P815 cells/eye. No evidence of tumor appeared in the injected eyes of any of these animals (Table I). This finding is consistent with the results of many previous studies (7) which document that a pre-existing state of alloimmunity robs the anterior chamber of its capacity to act as an immunologically privileged site for alloantigenic tissues.

TABLE I

Development of Local Tumors After Inoculation of P815 Cells into BALB/c Recipients

	Pretreatment	Site of P815* Inoculation	Tumors/eyes injected
1	None	Subcutaneous	0/10
2	None	Anterior chamber	20/20
3	DBA/2 Skin graft	Anterior chamber	0/20
4	P815 injected intracamerally	Subcutaneous	20/20 intracameral 10/10 subcutaneous

* 10^5 P815 cells inoculated 14 d after skin graft (line 3) or 5 d after intracameral injections (line 4).

TABLE II

Influence of Intracameral Inoculation of P815 Cells on the Alloimmune Response of BALB/c Mice to DBA/2 Minor Alloantigens

Primary exposure to DBA/2 alloantigens	*n*	Median survival time of subsequent DBA/2 skin grafts*
		d
1 None (first set)	10	10.8 (10.2–11.8)
2 DBA/2 skin allograft	10	7.0 (6.8–7.2)
3 P815 cells injected subcutaneously	10	7.0 (6.5–7.5)
4 P815 cells injected into anterior chambers of both eyes	8	>30‡

* Days + 95% confidence limits.

‡ Only two recipients rejected DBA/2 grafts; the remaining grafts were healthy throughout observation interval.

To verify that the alloantigens expressed on the P815 tumor were capable of inducing alloimmunity, normal BALB/c animals were injected subcutaneously with 10^5 P815 cells. 2 wk later, these mice received orthotopic DBA/2 skin grafts. These grafts were rejected briskly, with a median survival time of 7.0 d (Table II). This is identical to the accelerated rejection observed in BALB/c mice immunized with a primary DBA/2 skin graft (Table II). Thus, the P815 tumor cell line expresses DBA/2-specific transplantation antigens in a manner capable of eliciting specific alloimmunity. In an effort to understand the reason for the progressive growth of P815 cells in the anterior chamber of normal BALB/c mice, recipients of intracameral P815 tumor cell injections were grafted orthotopically 2 wk later with DBA/2 skin grafts. As might have been predicted from our previous experience with a similar model in rats (2, 5, 6), these animals proved to be relatively-to-absolutely unresponsive to DBA/2 alloantigens. The median survival time of DBA/2 skin grafts on BALB/c mice that bore the P815 tumor in the anterior chamber was >30 d (Table II). Six of eight animals failed absolutely to reject their DBA/2 skin grafts during the 30-d observation period.

Finally, BALB/c mice that were injected intracamerally on day 0 with P815 cells were injected subcutaneously 5 d later with 10^5 P815 cells. Progressively growing subcutaneous tumors developed in all of these animals (Table I). In control experiments, a genetically dissimilar tumor cell line, EL-4 (derived from the C57BL/6 strain), was unable to influence in any way the immune reactivity of BALB/c mice to DBA/2 alloantigens. Thus, the intracameral presentation of the minor histocompatibility antigens of DBA/2 expressed on P815 tumor cells subverted the capacity of recipient BALB/c mice to mount a typical immune response to DBA/2 minor histocompatibility antigens. Indeed, these recipients permitted extended survival of both skin and tumor allografts.

Discussion

Since their experimental description >30 yr ago (8), immunologically privileged sites have languished at the periphery of transplantation immunobiology. With relatively few exceptions, their investigation has been ignored by the immunological community. This relates partly to the fact that early studies ascribed the privileged state to the absence of lymphatic drainage routes (7), which allowed the simplistic

conclusion that antigens presented at sites of immunologic privilege escape detection by the immune system. More recent studies have indicated that this simple construction may be naive. In rats, when alloantigens (displayed on lymphocytes from semiallogeneic F_1 hybrids or on tiny skin allografts) are placed into the anterior chamber of the eye, they produce a state of immune deviation in which prolonged survival of subsequent orthotopic skin grafts is observed (2, 9). It was also found that this state of specific immune deviation depended upon: (*a*) the persistence of antigen within the anterior chamber, and (*b*) the presence of an intact and functional spleen (5, 6).

Further experimental analysis of the phenomenon in rats proved difficult because of limited availability of discriminating reagents and genetically defined allodisparate rat strains. As a consequence, the model was adapted for use in mice. The results reported in this communication are the first to emerge from this successful adaptation. These findings verify the validity and generality of the original hypothesis: antigens presented via the anterior chamber impact the immune response in an unusual fashion: as a consequence, the development of a destructive response is subverted; instead, a protective one is elicited. Although much needs to be done to understand the cellular and molecular basis of the phenomenon, it is already clear that there are likely to be important ramifications. We believe that immunologic privilege as described for the brain, the anterior chamber of the eye, the hamster cheek pouch, and, perhaps, other sites should no longer be regarded as an interesting, albeit unimportant, immunologic phenomenon. Rather, understanding the basis of this phenomenon holds considerable promise of yielding important information in the diverse fields of ocular immunology and transplantation biology.

Summary

The ability to introduce carefully controlled numbers of viable cells into the anterior chamber of mouse eyes made it possible to examine the interrelationship between presentation of antigens into the anterior chamber and into conventional body sites and their synergistic/antagonistic effects on the immune system. P815 mastocytoma (DBA/2; *H-2*^{d}) cells are syngeneic with BALB/c hosts at the major histocompatibility locus, but differ at multiple minor histocompatibility loci. When P815 cells were injected subcutaneously, they were rejected by BALB/c recipients who became specifically immune. By contrast, when P815 cells were injected intracamerally, they grew progressively into massive intraocular tumors; moreover, these BALB/c hosts proved subsequently unable to reject subcutaneously injected P815 cells, and, more impressively, failed to reject DBA/2 skin allografts placed orthotopically. Minor histocompatiblity antigens, presented first through the anterior chamber of mouse eyes, elicit a suppressive rather than an agressive host immune response that protects cells that bear these antigens from a destructive alloimmune reaction at both intracameral and systemic sites.

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