Interleukin-1 Abrogates Anterior Chamber-Associated Immune Deviation

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Alloantigens placed into the anterior chamber of the eye elicit antigen-specific suppression of systemic delayed-type hypersensitivity (DTH) responses and severe impairment of skin allograft rejection. This pattern of immunologic alteration has been termed anterior chamber-associated immune deviation (ACAID) and is the underlying mechanism responsible for immunologic privilege within the anterior chamber of the eye. Previous studies indicate that the immunologic privilege associated with the anterior chamber of the eye might be the result of a deficiency of interleukin-2 (IL-2) during antigen presentation. The present study examined the role of IL-1 in the induction of ACAID. It is well known that intracameral (IC) inoculation of DBA/2 mastocytoma cells (P815) into allogeneic BALB/c recipients results in antigen-specific suppression of DTH responses and progressive tumor growth. The authors found, however, that sublines of P388D1 (DBA/2 monocyte/macrophage tumor) that produce IL-1 not only do not grow progressively in the anterior chamber, but also they can prevent the suppression of DTH ($P < 0.01$). The role of IL-1 in the abolition of ACAID was confirmed in studies with IC-inoculated P815 cells. Systemic administration of exogenous IL-1 (by subcutaneous miniosmotic pumps) prevented the induction of ACAID in hosts that received IC inocula of P815 cells ($P < 0.01$). These results indicate that induction of ACAID and perhaps the immune-privileged character of the anterior chamber is dependent on an IL-1 deficiency during the processing of IC alloantigens.


The immune-privileged character of the anterior chamber of the eye has been well documented. Barker and Billingham described a 19th century observation in which xenogeneic tumor cells survived in the anterior chamber of a rabbit's eye, while similar cells did not survive at other body sites. The early explanation for this immunologic privilege to antigen in the anterior chamber was attributed to the lack of lymphatic drainage of the anterior chamber. It was believed that antigen was sequestered in the globe and, therefore, unable to be recognized by the host's immune system. More recently it has been shown that various antigens, administered into the anterior chamber, lead to specific antibody production, and normal cytotoxic T-lymphocyte (CTL) activity, but impaired skin allograft rejection and suppressed delayed-type hypersensitivity (DTH). This sequela is referred to as anterior chamber-associated immune deviation (ACAID), and has been documented after anterior chamber administration of Herpes simplex virus, haptenated syngeneic lymphoid cells, tumor-specific antigens, and minor histoincompatible tumor allografts.

P815 mastocytoma (DBA/2) cells are syngeneic with BALB/c at the major histocompatibility complex but differ at multiple minor histocompatibility loci. When inoculated into the anterior chamber, P815 grows progressively in allogeneic BALB/c hosts, experiencing immunologic privilege. The host undergoes normal antibody synthesis and CTL responses but with severely suppressed DTH responses and impaired skin allograft rejection. Other tumor studies have shown certain minor histoincompatible T-cell lymphoma cell lines do not grow progressively in the anterior chamber. In those studies interleukin-2 (IL-2) was shown to be sufficient for the abrogation of the immune deviation. We used the DBA/2 macrophage cell line P388D1, that is known to produce IL-1 and took advantage of two sublines of this tumor in an attempt to delineate further the mechanism of the DTH suppression induced by alloantigen administered into the anterior chamber.

Materials and Methods

Mice

Adult female BALB/c (H-2b), DBA/2(H-2d), C57BL/6 (H-2b) and C3H/HeJ mice were purchased
Tumor Cells

P815 mastocytoma cells (DBA/2 origin) were grown in suspension cultures in 75-ml culture flasks in Dulbecco's modified Eagle's minimal essential medium (DMEM; Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (FCS; Gibco) with 1% L-glutamine, 1% vitamin solution, 1% sodium pyruvate, and 1% streptomycin, penicillin, and amphotericin B (complete DMEM). CCL 46 (DBA/2) cells were obtained from American Type Culture Collection (ATCC; Rockville, MD) and were cultured in Minimum Essential Medium (Gibco) supplemented as mentioned (complete MEM). AD.4 cells, also a subline of CCL 46, were a generous gift from Donald A. Cohen, PhD (College of Medicine, University of Kentucky), and they were maintained as monolayer cultures in RPMI-1640 (Gibco) supplemented as mentioned, with the addition of 1% nonessential amino acids, 1% HEPES buffer, and the exclusion of vitamin solution (complete RPMI). All cells were incubated at 37°C and 5% CO2.

Anesthesia

For observation of tumor growth mice were lightly anesthetized by inhalation of methoxyflurane (Methofane; Pitman-Moore, Washington Crossing, NJ). For anterior chamber inoculation and footpad measurements, mice were deeply anesthetized with an intraperitoneal injection of 0.07-0.1 ml of a solution consisting of 31.7 mg/ml ketamine HCl (Ketalar; Parke-Davis, Morris Plains, NJ) and 4.8 mg/ml promazine HCl (Sparine; Wyeth, Philadelphia, PA) in phosphate-buffered saline.

Anterior Chamber Inoculations

The technique for transplanting tumor cells into the anterior chamber of the mouse eye has been described elsewhere.2 Tumor cells were suspended to a concentration of 1 X 10^5 cells in 5 μl of sterile Hank's balanced salt solution (HBSS) and inoculated intracameraly (IC). Beginning on day 12, eyes were examined every 2–3 days, and tumor growth documented.

Tumor Growth Assay

Tumor growth was scored as previously described.12 Using a dissecting microscope, anesthetized animals were observed, and tumor growth recorded as the percent of the anterior chamber occupied by tumor. Phtisical rejection was judged as atrophy and shrinkage of the globe concurrent with reduction of tumor size. Tumors were judged as cleanly rejected when no visible tumor was present in an intact, grossly normal globe.

DTH Assay

The DTH against relevant minor histoincompatible antigens was measured as footpad swelling as described previously.1 Mice received tumor cells administered either IC or subcutaneously (SC); 14–16 days later their footpads were challenged and measured for DTH responses. Both hind footpads of each mouse were measured with a Mitutoyo engineer's micrometer immediately before footpad challenge. An eliciting dose of 1 X 10^6 DBA/2 spleen cells or 1 X 10^5 P815 cells per footpad was inactivated by incubation in a 10% solution of mitomycin c (Sigma) in complete RPMI for 45 min at 37°C, washed four times, and resuspended in 25 μl of HBSS. Cells were then injected into the SC tissue of the right hind footpad. The left hind footpad served as a negative control and received 25 μl of HBSS without cells. Both footpads were measured 24 hr later, and the difference in footpad size was used as a measure of DTH. Results were expressed as specific footpad swelling = (24-hr measurement – 0-hr measurement) of the experimental foot – (24-hr measurement – 0-hr measurement) of the control foot.

Stimulation of IL-1 Production

The production of IL-1 was induced as previously described.13 Cell lines were cultured at a concentration of 1.5 X 10^6/ml media with 1 μg of phorbol 12-myristate 13-acetate (PMA; Sigma) per ml for 144 hr. Cell-free supernatants were obtained by centrifugation and tested for IL-1 using a thymocyte proliferation assay.

Thymocyte Proliferation Assay

A standard thymocyte proliferation assay was used as described.14 One hundred microliters of various dilutions of the experimental and control samples were incubated with 1.5 X 10^6/ml media with 1 μg of phorbol 12-myristate 13-acetate (PMA; Sigma) per well in 96-well plates with 100 μl of complete RPMI with 1 μg/ml of phytohemagglutinin (PHA-P; Sigma) for 72 hr at 37°C and 5% CO2. During the last 18 hr the cells were incubated with 1 μCi ^3H-thymi-
dine. Wells were then washed on a MASH II Automated Sample Harvester (Whittaker, Walkersville, MD). Incorporated thymidine was counted on a Beckman LS 3801 liquid scintillation counter (Ful- lerton, CA).

Exogenous IL-1 Administration

Commercially prepared cell-derived human IL-1 and recombinant IL-1 (rIL-1) were purchased from Genzyme (Boston, MA). Twenty units of either IL-1 or rIL-1 were loaded into sterile Alzet osmotic pumps (model 2001; Alza, Palo Alto, CA). The procedure for inserting the pumps has been described elsewhere. One unit (U) equals the amount of IL-1 required to double the proliferative response of mouse thymo-

Statistics

The student t-test was used to test the statistical validity of the data. Differences were considered significant when \( P \leq 0.05 \).

Results

Growth of Histoincompatible P388D1 Tumors in BALB/c Hosts

We previously reported that allogeneic tumor cells differing from the host at multiple minor histocompat-

![Fig. 1. Pattern of tumor growth in anterior chambers of BALB/c mice. Results are the average of five mice per group. *Hosts killed because of progressive tumor growth.](image1)

Abolition of ACAID by AD.4 Tumors and Restoration of DTH Responsiveness

One of the hallmarks of ACAID is the antigen-spe-

![Fig. 2. Growth of AD.4 in the AC of allogeneic BALB/c, and immunodeficient BALB/c nude hosts. n = 5 per group. *Hosts killed because of progressive tumor growth.](image2)

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![Fig. 3. Growth of AD.4 in the AC of allogeneic BALB/c, and immunodeficient BALB/c nude hosts. n = 5 per group. *Hosts killed because of progressive tumor growth.](image3)

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by CCL 46 is attributable to a diminished production of IL-1. The IL-1 production was stimulated by the addition of 1 μg/ml of PMA to the culture media of the various cell lines. Cells were cultured for 144 hr, and the supernatant was removed and assayed using a thymocyte proliferation assay. As predicted, the DBA/2 tumor line that abrogated ACAID also produced large quantities of IL-1 (Fig. 4). By contrast, CCL 46 cell cultures produced quantities of IL-1 not significantly different from that produced by P815 after stimulation with PMA. The results support the hypothesis that abrogation of ACAID, intraocular tumor rejection, and synthesis of IL-1 are closely correlated.

Exogenous IL-1 Abrogates ACAID

It is reasonable to assume that the P388D1 tumor lines elaborate other cytokines in addition to IL-1 and that these unidentified cytokines might participate in the abrogation of ACAID. Our assumption, however, was that IL-1 was the key element in the abrogation of ACAID by the AD.4 cell line. This was tested in the next series of experiments in which purified, cell-line derived IL-1 was administered systemically into mice primed in the anterior chamber with P815 mastocytoma cells. The results indicated that anterior chamber inoculation of P815 cells induced ACAID as shown by the conspicuous absence of DTH responses to DBA/2 alloantigens (Fig. 5). By contrast, systemic administration of 20 units of purified IL-1 abolished ACAID. The positive DTH responsiveness in IL-1-treated hosts was unrelated to the presence of a subcutaneous pump or the trauma induced by surgical procedures, since insertion of sham pumps did not abolish ACAID in hosts primed IC with P815 cells.

We strongly suspected that the ability of the AD.4 line of P388D1 to abolish ACAID was attributable to its secretion of abundant quantities of IL-1, while the feeble production of IL-1 by the CCL 46 tumor line permitted the induction of ACAID. Accordingly, the presence of exogenous IL-1 might prevent the induction of ACAID and promote the development of positive DTH responses in hosts primed IC with CCI 46 cells. This hypothesis was examined in the next series of experiments in which CCL 46 cells were inoculated IC into BALB/c hosts with miniosmotic pumps loaded with rIL-1. As shown in Table 1, hosts primed IC with CCL 46 cells and provided exogenous rIL-1 developed positive DTH responses to the DBA/2 alloantigens. The abolition of ACAID was antigen specific since similar hosts did not mount DTH responses to third-party C57BL/6 (B6) alloantigens (Table 1). Moreover, the administration of exogenous IL-1 alone did not provoke positive footpad swelling.

Fig. 3. Footpad swelling in response to day 14 challenge with DBA/2 splenocytes. Error bars represent SEM. n = 5 per group. NC = negative control; PC = positive control. *Statistically significant difference compared with NC. P < .02.

Fig. 4.3H-thymidine incorporation by C3H/HeJ thymocytes in response to cell-free supernatants. *Statistically significant difference as compared to P815 and CCL 46 groups. P < .01.

Fig. 5. Footpad swelling in response to day 14 challenge with P815 tumor cells, as described in “Materials and Methods.” Error bars represent SEM. n = 5 per group; NC = negative control; PC = positive control. *Statistically significant as compared to NC. P < .01.
Table 1. Specificity of DTH responses in BALB/c hosts treated with rIL-1

<table>
<thead>
<tr>
<th>Exp number</th>
<th>Priming*</th>
<th>rIL-1</th>
<th>Challenge</th>
<th>Specific swelling (mm × 10⁻³ ± SD)</th>
<th>P</th>
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<tr>
<td>1</td>
<td>None</td>
<td>–</td>
<td>DBA/2</td>
<td>26 ± 29</td>
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<td>DBA/2</td>
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<td></td>
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<tr>
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<td>CCL 46 SC</td>
<td>–</td>
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<td>&lt;.01</td>
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<td>B/6</td>
<td>1 ± 3</td>
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<tr>
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<td>B/6 SC</td>
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<td>157 ± 65</td>
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</table>

* Hosts were primed either IC or SC with 10⁵ CCL 46 tumor cells or 10⁶ C57BL/6 (B6) splenocytes. Footpads were challenged on day 14 with DBA/2 or B6 splenocytes as indicated. n = 5 per group.

Discussion

We previously proposed that the antigen-specific suppression of DTH that occurs as a consequence of ACAID is the result of an IL-2 deficiency during antigen presentation. However, the reason behind this deficiency has not been determined. It has been proposed that the antigen-specific unresponsiveness that occurs in neonatal tolerance is due to a deficit of IL-2 during antigen presentation and that neonatal tolerance can be broken by the systemic administration of IL-2. With this paradigm in mind, we suspected that a similar condition occurred with ACAID. This hypothesis was confirmed in studies in which exogenous rIL-2 was administered systemically by miniosmotic pumps after anterior chamber presentation of alloantigens. In the presence of exogenous IL-2, ACAID was abolished, and the hosts mounted normal DTH responses to the anterior chamber alloantigens. In the current study, we considered the possibility that the apparent IL-2 deficit that occurs in ACAID might be remedied by the administration of exogenous IL-1 which, in turn, would stimulate the synthesis of both IL-2 and IL-2 receptors by relevant antigen-specific T-cells responding to the intraocular alloantigens.

The results reported here support the hypothesis that IL-1 alone can redirect the immune response to ocular antigens from a suppressive pathway (ie, ACAID) to a normal outcome in which DTH responsiveness is restored. Although we can only speculate as to the mechanism behind the abolition of ACAID and the restoration of normal immune function, it is reasonable to suspect that exogenous IL-1 acted to stimulate the endogenous elaboration of increased quantities of IL-2. In a typical immune response to alloantigen, antigen presenting cells (APC) process antigen and present it to T-cells, while simultaneously secreting various cytokines, one of which is IL-1. The presentation of antigen stimulates the T-cell to express IL-2 receptors on its surface, while IL-1 triggers the T-cell to produce additional IL-2. The interaction of IL-2 with its receptors on the T-cell surface causes progression of the T-cells through the cell cycle and, hence, proliferation. This expansion of antigen-specific T-cells provides the host with an expanded population of antigen-specific, activated T-cells which proceed to stimulate various responses, including the DTH response. When IL-1 is not available, however, this process is thwarted, and in the case of the anterior chamber, antigen presentation occurs in a manner that favors down regulation of systemic DTH.

Why antigen processing in the anterior chamber leads to a down regulation of DTH is still mysterious. However, results from studies on the immunobiology of the skin may shed light on this conundrum. Granstein et al. found that ultraviolet (UV) irradiation destroys normal APCs in the skin and allows the emergence of UV-resistant epidermal cells which present antigen in a manner that induces suppression of systemic DTH. In this regard, it is intriguing that ACAID can be abrogated in BALB/c mice if Ia⁺ Langerhans cells are present in the cornea at the time of inoculation with mastocytoma cells or if host Langerhans cells are placed into the anterior chamber at the time of IC inoculation of P815 cells. This mosaic of findings suggests that if functioning APCs are present in the anterior chamber at the time of antigen deposition, ACAID will be bypassed, and normal systemic DTH reactivity will be induced. In light of the evidence that IL-2, Langerhans cells, and now IL-1 can abrogate ACAID, we propose that, under physiologic conditions, the anterior chamber has an inadequate number of the appropriate APCs, thereby leaving the task of antigen processing to secondary cells.
This is analogous to the UV-resistant, tolerogenic APC of the skin. The putative aberrant APC of the anterior chamber would in turn present alloantigens in a manner that promotes the development of ACAID.

The ability to circumvent ACAID with either IL-1, a cytokine closely associated with normal antigen presentation, or with exogenous Langerhans cells is consistent with the hypothesis that aberrant antigen processing is the underlying basis for immune privilege in the anterior chamber. If this hypothesis is confirmed, we will move one step closer to understanding the unique array of immunologic phenomena that occur in the eye.

Key words: ACAID, interleukin-1, ocular tumor, immune privilege

References