Propionibacterium Acnes-Enhanced Lens-Induced Granulomatous Uveitis in the Rat

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Propionibacterium acnes (Corynebacterium parvum) is being implicated more frequently as a cause of intraocular inflammation following cataract surgery. In addition to its role as an infectious agent, P. acnes also may possess adjuvant-like or adjuvant-enhancing properties. The presence of this organism in an eye with residual lens material after extracapsular cataract surgery could augment inflammation resulting from a phacoantigenic (phacoanaphylactic) response. We have modified an established rat model of lens-induced granulomatous uveitis (LIGU) to examine the adjuvant properties of P. acnes.

Our results suggest that P. acnes effectively potentiates LIGU. Invest Ophthalmol Vis Sci 33:1766-1770, 1992

Propionibacterium acnes is a well known modulator of the immune system in experimental animal models and in humans. In addition, it has been hypothesized that P. acnes may play a role in enhancing immune-mediated inflammation in the human eye. Recent reports have linked P. acnes with a syndrome of delayed-onset uveitis after extracapsular cataract extraction. Some of these cases of delayed-onset post-cataract surgery inflammation clinically resemble phacoantigenic uveitis, an antigen-specific immune-mediated inflammatory disease of the eye.

In humans, one form of phacoantigenic uveitis is phacoanaphylactic endophthalmitis, which presents histologically with a characteristic zonal granulomatous inflammation around the disrupted lens material. A virtually identical granulomatous intraocular inflammation can be produced by disrupting the lens capsule of Wistar rats that have been sensitized to soluble lens protein in Freund's complete adjuvant. To determine whether P. acnes could play a role in an immune-mediated (phacoanaphylactic) response to lens protein, we employed and modified the above rat model of lens-induced granulomatous uveitis (LIGU). We substituted P. acnes for the bacteria (Mycobacterium tuberculosis) in commercially prepared Freund's complete adjuvant (Sigma, St. Louis, MO). We compared the effectiveness of our P. acnes adjuvant preparation to that of complete Freund's adjuvant in the production of LIGU. Our studies focused on the histopathologic features that characterize LIGU in the rat.

Materials and Methods

Animals

We employed 67 male, outbred Wistar rats for our studies. All rats weighed between 200 and 225 g each at the beginning of the study. Our studies conformed to the ARVO Resolution on the Use of Animals in Research.

Immunizations

Rats were placed into one of three groups, each group containing 21-24 animals. All animals received four subcutaneous injections at 2 wk intervals. Each injection consisted of 1 cc containing 10 mg of soluble bovine lens protein emulsified with a different adjuvant preparation for each of the animal groups (Table 1). P. acnes was used (Group III) at a concentration of 1.0 mg/ml dry weight, equivalent to that of Mycobacterium tuberculosis in Freund's complete adjuvant (Sigma F-5881). One week following final injection, one lens capsule of each rat was disrupted with a 30 G needle through an anterior chamber approach. One week after lens disruption, eyes were removed for histopathologic evaluation.

Bacteria

P. acnes was obtained as a clinical isolate from the cerebral spinal fluid (CSF) of a patient with an in-
Table 1. Incidence of lens-induced granulomatous uveitis

<table>
<thead>
<tr>
<th>Group</th>
<th>Adjuvant preparation</th>
<th>N*</th>
<th>Incidence of LIGU†</th>
<th>Significance‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Lens protein in Freund's complete adjuvant§</td>
<td>21</td>
<td>85.7%</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>II</td>
<td>Lens protein in Freund's incomplete adjuvant†</td>
<td>24</td>
<td>25.0%</td>
<td>—</td>
</tr>
<tr>
<td>III</td>
<td>Lens protein and Heat-killed <em>P. acnes</em> in Freund's incomplete adjuvant¶</td>
<td>22</td>
<td>95.5%</td>
<td>$P &lt; 0.0001$</td>
</tr>
</tbody>
</table>

* Total number of animals per group.
† Lens-induced granulomatous uveitis determined by histopathologic evaluation.
‡ Comparisons of Groups I and III to Group II, $P$ value for incidence of LIGU determined by chi-square analysis.

§ Adjuvant preparation contains *M. tuberculosis*.
† Adjuvant preparation without *M. tuberculosis*.
¶ *P. acnes* substituted for *M. tuberculosis* in the adjuvant preparation.

Incidence of lens-induced granulomatous uveitis

Table 1 presents the results of histopathologic evaluation. The Group III animals immunized with lens protein, *P. acnes*, and Freund's incomplete adjuvant (Group III) presented with the highest incidence of LIGU. The disease was present in 21 of 22 Group III rats and in 18 of 21 Group I rats. Only 6 of 24 animals in Group II exhibited features consistent with LIGU. Chi-square analysis revealed that Groups I and III had a significantly higher incidence of LIGU ($P < 0.0001$) than Group II. There was no significant difference in the incidence of disease between Groups I and III ($P < 0.27$).

Histopathology

All enucleated eyes were fixed in 4% formaldehyde, processed routinely, and embedded in paraffin for sectioning. Midsagittal, 8 µm serial sections were taken through each globe and stained with hematoxylin and eosin. Each eye was evaluated for granulomatous inflammation around the lens using the criteria established by Marak, et al. The globes were examined at the site of capsular disruption for a zonal infiltrate of polymorphonuclear (PMN) leukocytes invading the lens, surrounded by macrophages and epithelioid cells, and the presence of multinucleated giant cells. The extent of the infiltrate (severity) varied. However, eyes exhibiting the above histopathologic characteristics were considered positive for LIGU. A negative response was characterized by an infiltrate of PMN leukocytes (acute phakitis) or macrophages only.

Data Analysis

Histopathologic evaluation was performed by an eye pathologist who did not know the correlation between the tissues and the study groups. Each eye was assessed as positive or negative for LIGU. Comparisons of the incidence of LIGU for each group was performed by chi-squared analysis.

Results

Histopathologic examination of eyes exhibiting LIGU revealed an admixture of an acute and granulomatous inflammatory cell response at the site of lens capsule disruption. This was characterized by a zonal inflammatory infiltrate consisting of PMN leukocytes invading the disrupted lens cortex. This zone of inflammatory cells was surrounded by macrophages, epithelioid cells, and multinucleated giant cells (Fig. 1). Animals not exhibiting LIGU presented with a non-specific inflammatory response composed of PMN leukocytes (acute phakitis) or macrophages only.

Bovine Lens Protein

Bovine lens protein was prepared aseptically by crushing whole bovine lenses in cold normal saline (10 lenses/100 ml saline), twice passing the suspension through a 500 µm pore size stainless steel screen, and centrifuging at 500–800 rpm at 4°C for 5 min. Following a BioRad protein assay (BioRad Laboratories, Richmond, CA), the clear supernate was divided into 5 ml aliquots and stored at −20°C.

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Fig. 1. Histopathologic comparison of rat models of lens-induced granulomatous uveitis (LIGU). Note the typical zonal granulomatous reaction made up of an inner zone of polymorphonuclear leukocytes (arrows), multinucleated giant cells (curved arrows), and an outer zone of epithelioid cells (arrowheads). Note the presence of lens material (N). (A) Group I rats sensitized using lens protein and Freund's complete adjuvant. (B) Group III rats sensitized using lens protein, P. acnes, and Freund's incomplete adjuvant show similar but more prominent epithelioid response (hematoxylin and eosin, x280).
**Discussion**

*Propionibacterium acnes* has been shown to have a variety of different immunogenic properties, including potentiation of the humoral immune response,

1,10 potentiation or modulation of cell mediated hypersensitivity,

3,20 and cytotoxicity,

2,5,9,11,21 activation of macrophages,

22 and activation of the complement cascade.23,24 In addition, *P. acnes* has been reported to stimulate the reticuloendothelial system

4,25,26 and induce interferon.27 The role of *P. acnes* in ocular inflammation is not well understood. *P. acnes* is a member of the external eye flora,28 and inadvertent inoculation of the organism into the eye could occur during surgery, particularly during intraocular lens insertion. Once in the eye, the organism may remain for extended periods because it has been shown to be resistant to degradation by reticuloendothelial cells and their lytic enzymes.29–31 We have found the organism in eyes undergoing intraocular lens removal at the time of keratoplasty for pseudophakic bullous keratopathy.32 Prolonged intraocular sequestration of *P. acnes*, in association with residual lens protein, may result not only in a chronic, low-grade, infectious process, but also in an immunopathogenically mediated disease such as phacoanaphylactic endophthalmitis. We used a modified rat model of LIGU to investigate the possibility that this organism could function as an adjuvant in an immunopathogenic process in the eye. Our results demonstrate that *P. acnes* may exhibit adjuvant-like or adjuvant-enhancing properties, comparable to those of the *Mycobacterium* in Freund’s complete adjuvant, in the production of experimental LIGU, as determined by histopathologic features.

While much remains to be learned about the precise role of *P. acnes* in human postoperative ocular inflammation, the present study lends support to the hypothesis that in the presence of residual lens protein this organism has the potential to produce or enhance an immunogenic-like response that histologically resembles phacoanaphylactic endophthalmitis.

**Key words:** *Propionibacterium acnes*. adjuvant, lens-induced granulomatous uveitis, rat

**References**


20. Maguire HC Jr, and Cipriano D: Immunopotentiation of cell-mediated hypersensitivity by *Corynebacterium parvum* (Pro-


