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Ocular delayed hypersensitivity: A pathogenetic mechanism of chlamydial conjunctivitis in guinea pigs

(chlamydial group antigen/immunology/disease pathogenesis)

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ABSTRACT **We used a naturally occurring, *Chlamydia psittaci*-caused eye disease in guinea pigs, guinea pig inclusion conjunctivitis, as an animal model to understand both the immune response and the pathogenesis of chlamydial eye infections. When instilled into the conjunctival sac of guinea pigs that had been previously infected and were immune, viable chlamydiae or a Triton X-100-soluble extract of them produced a short-lived (12–48 hr) eye disease indistinguishable clinically and histologically from that observed during primary chlamydial eye infection. The clinical and histologic findings were consistent with those of ocular delayed hypersensitivity. Ocular delayed hypersensitivity was induced by primary chlamydial infection at mucosal sites other than conjunctival, such as vaginal and intestinal. Preliminary characterization of the hypersensitivity allergen shows that it is heat sensitive and common to the genus *Chlamydia*. The allergen is apparently not surface-exposed on chlamydiae and requires viable but not replicating organisms for activity. Our observation should be useful in understanding pathogenetic mechanisms of *Chlamydia trachomatis*-caused infections in humans, in particular those that produce chronic inflammatory diseases, such as blinding trachoma and urogenital diseases.**

Blinding trachoma is the result of chronic inflammation of the conjunctiva caused by infection with *Chlamydia trachomatis*, an obligate intracellular parasite. This preventable disease is a significant public health problem in developing countries, where it afflicts about 500 million persons, of whom 7 million are blind (1). Primary infection results in a self-limiting mucopurulent follicular conjunctivitis that normally resolves without complicating sequelae. Protective immunity against infection is short-lived. Upon reinfection, the disease progresses to a more severe form of trachoma that is characterized by chronic follicular keratoconjunctivitis followed by neovascularization of the corneal stroma, clinically manifested as pannus (2). It is not clear if repeated infection, persistent infections, or ocular hypersensitivity influence the progression of the disease toward blinding trachoma. In a nonhuman primate model (3, 4) or in a guinea pig model (5, 6), repeated exposure to chlamydiae has been shown to produce chronic disease characteristic of trachoma. Chlamydiae were rarely isolated from the conjunctiva during chronic disease, suggesting that trachoma pathogenesis is an immunologically mediated response (i.e., ocular hypersensitivity). However, these studies failed to distinguish between active chlamydial infection and hypersensitivity to chlamydial components.

Because a practical *C. trachomatis* animal model for trachoma is not available, we studied the naturally occurring *Chlamydia psittaci* eye infection in guinea pigs, guinea pig inclusion conjunctivitis (GPIC) (7–12), in attempts to understand the host's immune response in the pathogenesis of chlamydial eye disease. We describe here an ocular delayed hypersensitivity induced in guinea pigs following resolution of primary GPIC. The inflammation elicited by a soluble chlamydial allergen was clinically and histologically indistinguishable from that of the primary infection, demonstrating that the pathogenesis of this ocular disease is immunologically mediated.

MATERIALS AND METHODS

Organisms. The *C. trachomatis* serovar H, strain UW-4/CX, and serovar B, strain TW-5/OT, and the *C. psittaci* strains meningopneumonitis Cal-10 (Mn) and GPIC were grown in HeLa 229 cells and elementary bodies (EBs) purified on Renografin gradients (13). Inclusion-forming units (IFUs) were determined by methods described previously (14). *Neisseria gonorrhoeae* strain JS3 was grown in gonococcal Hepes broth (15). *Escherichia coli* strain JM109 was grown in LB media supplemented with 1 mM isopropyl-α-D-thiogalactopyranoside and 250 μg of carbenicillin per ml (16).

Hypersensitivity Test Allergens. Chlamydial EBs (10^9 IFU), *E. coli* (10^9 cells), or *N. gonorrhoeae* (10^9 cells) were incubated in 1 ml of 0.5% Triton X-100 in phosphate-buffered saline (0.15 M NaCl/0.025 M sodium phosphate, pH 7.4) for 30 min at 37°C. Whole organisms and macromolecular complexes were removed by centrifugation at 100,000 × *g* at 4°C for 1 hr. The resulting Triton X-100-soluble extracts (TX-100 extracts) were used as hypersensitivity test allergens. The protein concentrations of the TX-100 extracts were determined by using the Bio-Rad protein assay system with bovine serum albumin as a standard. To partially characterize properties of the allergen from GPIC EBs that elicits hypersensitivity, GPIC EBs or the TX-100 extract of them were treated by heat application, formalin-fixation, or UV-irradiation. GPIC EBs and the TX-100 extract from them were heat-treated by incubating at 56°C for 30 min. Formalin-fixed EBs were prepared by incubating EBs (10^9 IFU/ml) with 0.2% formalin in phosphate-buffered saline overnight at 4°C. The EBs were then pelleted and resuspended in phosphate-buffered saline. GPIC EBs (10^9 IFU/ml) were irradiated with a Sylvania germicidal UV lamp at 10 cm for 30 sec at room temperature. As assessed by infectivity titrations in HeLa 229 cells, the heat-treated and the formalin-fixed EBs were nonviable, whereas 0.01% of the UV-irradiated GPIC EBs were viable. Thus, a challenge dose of 10^6 IFU of UV-irradiated organisms contained 1.3×10^2 viable IFU, enough to cause infection in previously uninfected animals but not to elicit hypersensitivity in immune animals. UV-irradiated organisms were extracted with 0.5% Triton X-100 in phos-

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Abbreviations: GPIC, guinea pig inclusion conjunctivitis; EBs, elementary bodies; LPS, lipopolysaccharide; IFU, inclusion-forming units; TX-100 extract, Triton X-100-soluble extract.

phate-buffered saline as described above. The TX-100 extract of viable organisms was UV-irradiated as described above for chlamydial organisms.

Chlamydial Infections of Guinea Pigs. Female Hartley guinea pigs (age 6–8 wk) from a chlamydial-free colony (Rocky Mountain Laboratories) were used in all experiments. During experimental procedures, animals were briefly anesthetized (6.25 mg of Ketamine and 1.25 mg of Xylazine per kg of body weight). Animals were infected conjunctivally with $5\times$ ID_{50} (5×10^2 IFU) of *C. psittaci* strain GPIC by instilling 10 μl of the chlamydial suspension directly into the lower conjunctival sac. Conjunctivitis appeared in 3–4 days and resolved in 14–20 days. Female guinea pigs were infected vaginally by injecting 1×10^6 IFU into the vagina. At indicated time, swabs (Calgiswabs type 1, Spectrum Laboratories, Houston, TX) were applied to the conjunctival or vaginal mucosa or both and cultured on HeLa cell monolayers, and the number of IFU recovered was determined as described above. Six weeks after vaginal or conjunctival infection was initiated (3–4 weeks after the disease resolved), animals were tested for ocular or cutaneous delayed hypersensitivity or both. Animals were infected intestinally by injecting 1×10^7 IFU directly into the duodenum distal to the pyloric sphincter during midline laparotomy. Most animals had a mild enteritis, with diarrhea of 1–3 days duration and some weight loss. No attempt was made to culture chlamydiae from feces. Four weeks later, animals were tested for hypersensitivity with the TX-100 extract of GPIC EBs and for immunity to conjunctival challenge with GPIC organisms.

Intramuscular Immunization of Guinea Pigs. Guinea pigs were immunized intramuscularly with 2×10^8 IFU of *C. psittaci* strain GPIC in Freund's incomplete adjuvant on days 0, 14, and 21. One week after the last injection, animals were tested for hypersensitivity and immunity to conjunctival challenge with GPIC agent.

Delayed Hypersensitivity. Ocular delayed hypersensitivity was elicited by instilling equal protein concentrations of the appropriate challenge allergen (10–50 μl) into the lower conjunctival sac. In general, each eye received a single dose of test allergen. The conjunctival response was assessed clinically at 2, 18, 24, and 48 hr and was scored on a scale of 1–4. (1, slight hyperemia and edema of the lower palpebral conjunctiva; 2, hyperemia and edema of the lower palpebral conjunctiva with slight hyperemia of the bulbar conjunctiva; 3, overt hyperemia and edema of the lower palpebral and bulbar conjunctiva; and 4, same as 3, with the addition of a mucopurulent exudate.) The cutaneous delayed hypersensitivity response to chlamydial allergens was measured 24 hr after intradermal injection of 100 μl of the test material (0.35 μg of chlamydial protein in the TX-100 extract or a Triton X-100/phosphate-buffered saline control) over the backs of guinea pigs. The buffer control was negative in each animal. The diameter of erythema and the degree of induration were measured. The response was considered strong when the diameter of erythema was greater than 15 mm, medium when between 10 and 15 mm, weak when between 5 and 10 mm, and negative when less than 5 mm.

Histology. The upper and lower eyelids from each eye were removed in one elliptical piece, stuck flat on cardboard conjunctival surface down, and fixed in neutral-buffered 10% formalin. The globes were freed of orbital soft tissues and fixed whole in Zenker's fluid. Vertical paraffin sections of the eyelids and globes were stained with hematoxylin and eosin.

RESULTS

Guinea Pig Inclusion Conjunctivitis Is an Immunologically Mediated Disease. The clinical appearance and histologic findings of a guinea pig eye with GPIC are shown in Fig. 1 *B* and *B'*. The main clinical signs were edema and hyperemia of both the upper and lower palpebral and bulbar conjunctiva accompanied by a mucopurulent exudate. Clinical signs first appeared 3–4 days after inoculation of chlamydiae and persisted for about 14 days, at which time the conjunctivitis resolved without complicating sequelae. Histological findings of the conjunctiva at 4 and 7 days after inoculation showed the edematous propria of the palpebral conjunctiva was heavily infiltrated with lymphocytes and larger mononuclear cells (Fig. 1*B'*). A few neutrophils (pseudoeosinophils) infiltrated the conjunctival epithelium, which sometimes was disrupted with loss of epithelial cells. The mononuclear cell infiltrate extended into the bulbar conjunctiva and contiguous limbus, but the cornea was spared.

Four weeks after resolution of a primary infection, animals were challenged in the lower conjunctival sac with either $10^4 \times ID_{50}$ (10^6 IFU) of GPIC agent or a TX-100 extract of viable EBs. In these animals, the inflammatory response (Fig. 1*C*) was indistinguishable clinically from that found in animals with primary GPIC (Fig. 1*B*). Signs of acute inflammation appeared at 12 hr, peaked between 18 and 36 hr, and was completely resolved by 72 hr after inoculation. This short-lived inflammatory response was not observed in animals that had not been infected previously with GPIC organisms. Triton X-100 buffer alone did not elicit an inflammatory response in either immune or naive guinea pigs. The cellular infiltrate in the conjunctiva was similar to that seen during primary infection (7 days postinoculation) in that the infiltrate was composed of lymphocytes and larger mononuclear cells (Fig. 1*C'*). The only significant histologic difference was an added eosinophilic infiltration of the limbus in animals that received GPIC TX-100 extract. The time course of the inflammatory response and the nature of the cellular infiltration is consistent with delayed hypersensitivity. Hypersensitivity did not occur in immunologically naive guinea pigs or in immune animals challenged with less than $10^4 \times ID_{50}$ of GPIC EBs (Table 1). Immune guinea pigs challenged with $10^4 \times ID_{50}$ or with the TX-100 extract were culture-negative for GPIC organisms at both 24 hr and 5 days after challenge. Thus, the inflammatory response was mediated by chlamydial antigen(s) and was not the result of chlamydial infection.

Ocular Delayed Hypersensitivity Can Be Elicited by Infection at Mucosal Sites Other than the Conjunctiva. The agent of GPIC naturally infects the urogenital and intestinal tracts as well as the conjunctiva of the guinea pig (8–10). Ocular delayed hypersensitivity to the TX-100 extract of GPIC EBs was induced by primary vaginal or intestinal infection (Table 2). After a primary conjunctival or vaginal infection, strong hypersensitivity responses occurred with mean clinical scores of 3.6 and 3.4, respectively. A more moderate response (mean clinical score of 2.4) was elicited after intestinal infection. Systemic immunization (intramuscularly) with viable GPIC EBs induced an extremely weak ocular hypersensitivity in half the animals (clinical score of 1.1) and no response in the remainder. These findings indicate that ocular hypersensitivity is most effectively induced by primary infection of mucosal surfaces—i.e., conjunctival, vaginal, or intestinal.

Cutaneous delayed hypersensitivity has been reported after either natural infection or systemic immunization with GPIC organisms (11, 17). Guinea pigs that had recovered from conjunctival, vaginal, intestinal, or systemic infection were tested for cutaneous hypersensitivity (Table 2). A cutaneous delayed hypersensitivity occurred in all animals previously infected with GPIC organisms. After conjunctival, genital, or intestinal infection, the mean diameters of erythema were 12.1 ± 2.0, 8.6 ± 3.1, and 9.9 ± 3.5, respectively. A much more intense response (diameter = 19.4 ± 3.0) occurred in animals that had been sensitized by intramuscular immunization. In these animals, the cutaneous response resulted in necrosis of the test site, indicating extreme sensitivity to the allergen. Thus, in systemically immunized animals, weak ocular hypersensitivity and strong

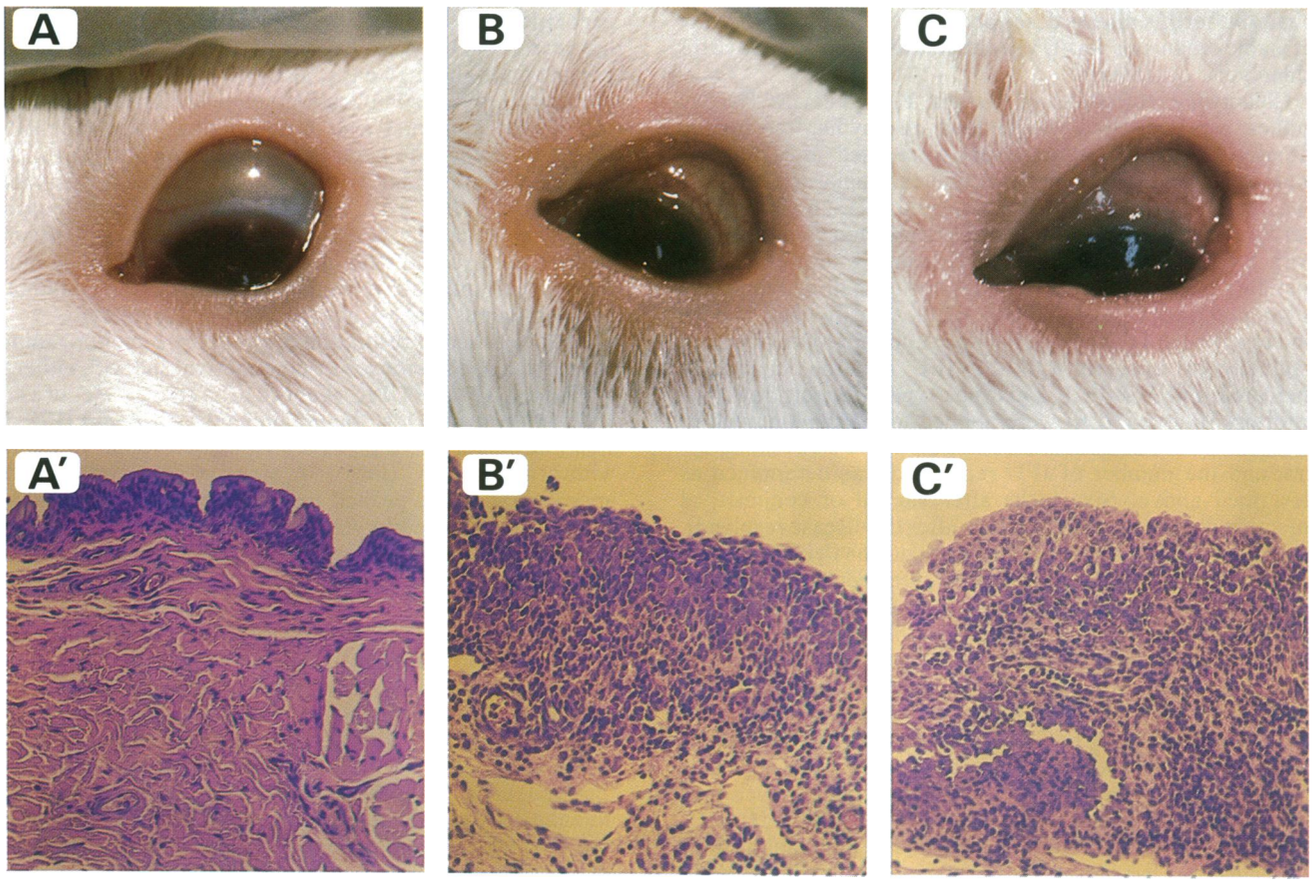

FIG. 1. Appearance of normal guinea pig eye (*A*), guinea pig eye 7 days after infection with the GPIC agent (*B*), and immune guinea pig eye 24 hr after challenge with the TX-100 extract of GPIC EBs (*C*). Hematoxylin- and eosin-stained sections of the palpebral conjunctiva: normal palpebral conjunctiva of a noninfected guinea pig (*A′*), palpebral conjunctiva of a guinea pig 7 days after inoculation with *C. psittaci* (*B′*) (the histologic findings 4 days after inoculation were similar to those observed 7 days after inoculation), and palpebral conjunctiva of an immune guinea pig 24 hr after challenge with a TX-100 extract of GPIC EBs (*C′*).

cutaneous hypersensitivity were observed with the same challenge preparation. The finding shows that cutaneous hypersensitivity is not necessarily indicative of hypersensitivity in other tissues.

The degree of protective immunity to conjunctival infection varied among groups of animals after systemic immunization or infection of the conjunctiva, vagina, or intestine (Table 2). After recovery from a primary conjunctival infection, all animals were solidly immune when challenged with $5 \times ID_{50}$ of GPIC agent. These animals remained free of clinical conjunctivitis, and chlamydiae could not be cultured from their conjunctivae 5 days postchallenge. Primary intestinal infection produced intermediate immunity to conjunctival infection. Guinea pigs in this group had minimal clinical disease at 5 days postchallenge, and 7×10^3 fewer IFU were recovered than were recovered from control animals. Disease in animals that had recovered from a primary vaginal infection or that had been systemically immunized was also less severe and of shorter duration than that observed in controls; however, similar numbers of chlamydiae were recovered from the conjunctivae of control and challenged animals. Although based on limited numbers of animals, these data indicate that solid protective immunity was only achieved by experimental infection of the conjunctiva. Of the three other routes of infection or immunization, intestinal infection afforded the greatest protection, although a limited

Table 1. Induction of ocular delayed hypersensitivity by viable EBs or a TX-100 extract of GPIC EBs

Challenge GPIC preparation	Clinical disease,* no. positive/no. tested		Isolation of Chlamydiae,† mean IFU recovered			
			Immune		Naive	
	Immune	Naive	24 hr	5 days	24 hr	5 days
Viable EBs						
$5 \times ID_{50}$ (5×10^2 IFU)	0/8	0/8	0	0	0	2×10^6
$10^4 \times ID_{50}$ (1×10^6 IFU)	8/8	0/8	0	0	6×10^3	2×10^6
TX-100 extract‡	8/8	0/8	0	0	ND	ND

Immune animals were tested 4–5 wk after resolution of primary ocular infection. Naive animals had not been infected with *C. psittaci*.

*Clinical disease was assessed 24 hr postchallenge and was scored as described. A clinical score of 2 was considered positive.

†Chlamydiae were isolated from the conjunctiva 24 hr and 5 days postchallenge.

‡The equivalent of 7 μg of protein was instilled into the lower conjunctival sac; ND, not determined.

Table 2. Ocular delayed hypersensitivity depends on immune stimulation at mucosal surfaces

Route of infection or immunization*	Delayed hypersensitivity†				Immune status to ocular GPIC challenge‡			
	Ocular		Cutaneous					
	No. positive/ no. tested	Clinical score	No. positive/ no. tested	Diameter of erythema	No. tested	Isolation of *Chlamydia*	Clinical score	Duration of clinical disease, days
Ocular	8/8	3.6 ± 0.5	8/8	12.1 ± 2.0	4	0	0	0
Genital	9/9	3.4 ± 0.8	4/4	8.6 ± 3.1	5	4.4×10^5	2.2 ± 0.95	4–8
Intestinal	4/5	2.4 ± 1.1	2/3	9.9 ± 3.5	4	8.0×10^2	1.0 ± 0	2–3
Systemic	3/6	1.1 ± 0.9	6/6	19.4 ± 3.0	6	4.7×10^5	1.3 ± 0.47	3–5
Naive§	0/8	0	0/8	0	8	1.8×10^6	4.0 ± 0	10–16

*Animals were infected or immunized as described.
†Guinea pigs were challenged conjunctivally with 20 μl (7 μg of protein) of the TX-100 extract of GPIC EBs. Ocular hypersensitivity was assessed 24 hr after challenge and scored as described. The mean clinical score (±SD) is listed. Animals were challenged intradermally with 100 μL (0.35 μg of protein) of a 1:100 dilution of the TX-100 extract of GPIC EBs. Erythema and induration were measured 24 hr later.
‡Guinea pigs were challenged with 5 × ID_{50} of the GPIC agent. Cultures for chlamydiae were done 5 days after infectious challenge, and the mean number of IFUs recovered at this time is listed. Clinical disease, when observed, first appeared 3–4 days after infectious challenge. Clinical disease was assessed daily, and protective immunity was evaluated by the intensity of disease (with the mean clinical score at 5 days listed) and duration (in days) of clinical disease.
§Animals with no previous exposure to GPIC.

degree of protection was observed after vaginal infection or intramuscular immunization. Similar results have been described by Nichols and co-workers (7–9, 18), who demonstrated protective immunity in the conjunctiva after either conjunctival infection or enteric (oral) immunization. They also demonstrated partial protective immunity in the conjunctiva after vaginal or urethral infection. Their results were similar to our findings of shortened duration and decreased clinical severity of the challenge infection.

Ocular Delayed Hypersensitivity Is Elicited by a Genus-Specific, Heat-Labile Chlamydial Allergen. To investigate the specificity of the hypersensitivity allergen, TX-100 extracts of *C. trachomatis* serovars B and H, *C. psittaci* strain Mn, *E. coli*, and *N. gonorrhoeae* were instilled in the eyes of immune and naive guinea pigs (Table 3). TX-100 extracts were standardized for protein content, and the equivalent of 7 μg of protein was tested for each organism. All TX-100 extracts of chlamydiae elicited ocular delayed hypersensitivity in GPIC immune guinea pigs, whereas TX-100 extracts of *E. coli* JM109 or *N. gonorrhoeae* JS3 did not. This suggests that the allergen is common in and restricted to the genus *Chlamydia*.

GPIC EBs elicited hypersensitivity only when viable (Table 3). Heat-killed, formalin-fixed, and UV-irradiated EBs were incapable of doing so. Like the intact EB, the allergen in the TX-100 extract was sensitive to heat; however, UV-irradiation of the TX-100 extract did not inactivate the allergen. The chlamydial allergen was extracted from UV-irradiated EBs, which by themselves were incapable of eliciting hypersensitivity. Thus, UV-irradiation does not destroy the hypersensitivity allergen but may prevent it from being expressed by the UV-irradiated organisms. This finding indicates that the chlamydial allergen may not be surface-exposed and requires a UV-sensitive event for expression. Extraction with Triton X-100 circumvents this mechanism.

Lipopolysaccharide (LPS), which has been described as a genus-specific heat-stable antigen (20), is the major component in the TX-100 extract. Phenol/water-purified LPS (20) in phosphate buffered saline either alone or with 0.5% Triton X-100 did not elicit hypersensitivity (Table 3). In addition, an *E. coli* recombinant (19) expressing the genus-specific LPS epitope did not elicit hypersensitivity.

DISCUSSION

The data presented here suggest that the pathogenesis of chlamydial disease is mediated by delayed hypersensitivity. Our conclusions are based on the observation: a soluble extract of viable chlamydial EBs inoculated into the conjunctival sac of immune animals produced an ocular disease indistinguishable clinically and histologically from primary GPIC. Although we used a *C. psittaci* strain in these studies, we have described recently similar findings with a *C. trachomatis* strain in a nonhuman primate model of blinding trachoma (21). In view of our findings, the pathogenesis of chronic or recurrent chlamydial conjunctivitis may be mediated by ocular hypersensitivity to chlamydial allergens and may not be due to active infection.

Identifying hypersensitivity as a major pathogenetic mechanism is important not only in understanding chlamydial-

Table 3. The hypersensitivity allergen is heat-sensitive, genus-specific, and subsurface

Organism*	Clinical disease,† no. positive/ no. tested	
	Immune	Naive
C. psittaci TX-100 extract		
GPIC	8/8	0/8
Mn strain Cal-10	6/6	0/2
C. trachomatis TX-100 extract		
UW-4/CX, H serovar	4/4	0/2
TW-5/OT, B serovar	6/6	0/2
E. coli TX-100 extract	0/6	0/6
N. gonorrhoeae TX-100 extract	0/4	0/2
TX-100 control‡	0/8	0/8
GPIC EBs§		
$10^4 \times ID_{50}$ (56°C, 30 min)	0/4	0/4
$10^4 \times ID_{50}$ (formalin-fixed)	0/4	ND
$10^4 \times ID_{50}$ (UV-irradiated)	1/4	0/2
TX-100 extract of GPIC§		
TX-100 extract (56°C, 30 min)	0/4	0/4
TX-100 extract (UV-irradiated)	6/6	0/2
TX-100 extract of UV-irradiated EBs	6/6	0/2
Purified GPIC LPS¶	0/8	0/6

ND, not determined; Mn, meningopneumonitis.
*Purified chlamydial EBs were extracted with 0.5% Triton X-100 in phosphate-buffered saline as described. The protein concentration of each extract was adjusted to 140 μg/ml, and a 50-μl aliquot was placed in the lower conjunctival sac.
†Immune status and testing were done as described in Table 1.
‡A 50-μl aliquot of 0.5% Triton X-100/phosphate-buffered saline was placed in the lower conjunctival sac.
§GPIC EBs or the TX-100 extract were treated as described.
¶GPIC LPS was purified by phenol/water extraction (19).

associated disease processes but also in establishing a foundation for future strategies to control chlamydial diseases by immunoprophylaxis. For example, Grayston and co-workers (22–25) have argued strongly that hypersensitivity is the mechanism responsible for blinding trachoma. Their conclusions were based on observations gathered during their pioneering efforts to develop a trachoma vaccine for use in humans. For example, in trachoma vaccine trials in monkeys, systemic immunization with highly potent vaccine preparations resulted in short-lived immunity specific for the immunizing trachoma serovar. When immunity waned, however, reinfection often produced more severe clinical disease than that observed in unvaccinated controls. In addition, challenge with a heterologous serovar often induced more severe clinical disease. From those studies, they concluded that protective immunity was serovar specific, whereas a deleterious hypersensitivity response, observed after protective immunity waned or in animals challenged with heterologous serovars, was directed against a "nonspecific" chlamydial antigen.

Our findings are particularly relevant to those early studies. Our data demonstrate that the pathogenesis of GPIC is mediated by delayed hypersensitivity to an allergen common to strains of both chlamydial species. Thus, the deleterious effect observed in the vaccine trials of Grayston and coworkers might be attributed to hypersensitivity elicited by the genus-specific allergen we have described here. From our observations and from those described above, we propose that the hosts' immune response to chlamydial infections consists of both protective and deleterious elements. The protective response is directed against antigens unique to particular strains or serovars; in contrast, the deleterious response is directed against a shared allergen that may be similar to or identical with the "nonspecific" antigen described in the vaccine studies.

We have shown that ocular delayed hypersensitivity is induced by chlamydial infection at mucosal surfaces other than conjunctival—i.e., intestinal or vaginal. Primary chlamydial infection at one mucosal site can elicit hypersensitivity reaction at either the same or different mucosal surfaces and may contribute to the pathogenesis of chlamydial disease in humans. In the absence of protective immunity, repeated oculogenital *C. trachomatis* infections may result in more severe or chronic episodes of cervicitis, urethritis, or more extensive *C. trachomatis* infections of the urogenital tract.

Although we have not identified the allergen eliciting hypersensitivity, we have characterized it as being heat sensitive and genus specific. To date, two chlamydial group antigens have been partially characterized with respect to function and chemistry. A complement-fixing, heat-resistant, periodate-sensitive antigen has been described (26, 27). This determinant is located on chlamydial LPS (20). The other group antigen is a heat-sensitive antigen with hemagglutinating properties (28, 29). LPS is the major component in the TX-100 extract; nevertheless, purified LPS did not elicit hypersensitivity in immune animals. This finding suggests that LPS is not the allergen; however, it does not preclude the allergen being composed of a complex of LPS with protein, carbohydrate, or lipid. The relationship of the hypersensitivity allergen to the chlamydial hemagglutinin is not known; however, they do share similar properties of being heat sensitive and genus specific.

Although the etiologic agent of GPIC is a strain of *C. psittaci*, the conjunctivitis and the urogenital and intestinal infections of the guinea pig are clinically similar to those caused by *C. trachomatis* in humans. Thus, the guinea pig model should be useful in identifying hypersensitivity allergens and potential vaccine candidates for trachoma and other chlamydial diseases.

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