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Antichlamydial Specificity of Conjunctival Lymphocytes during Experimental Ocular Infection

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Antigen-specific responses to chlamydiae have been demonstrated with lymphocytes isolated from the conjunctiva after primary ocular infection and after topical challenge of chlamydia-immune cynomolgus monkeys with noninfectious, Triton X-100-extracted antigen. Proliferative responses to viable elementary bodies homologous to the original infecting serovar were demonstrated. In addition, in vitro production of antichlamydial antibody by conjunctival B cells was demonstrated by enzyme-linked immunosorbent assay of culture supernatants collected after 7 to 21 days of culture. These findings demonstrate that antigen-specific lymphocytes appear in the conjunctiva as a result of ocular chlamydial infection and that a noninfectious chlamydial antigen stimulates their reappearance or expansion at the site of original infection.

Trachoma is a leading cause of infectious blindness in the world (6, 9) and results from chronic conjunctival infection by *Chlamydia trachomatis*. In recent years, a cynomolgus monkey model of trachoma has been used to investigate local and systemic immune responses to chlamydiae during acute and chronic ocular infection (3, 21–24, 29, 30). To date, long-lasting effective immunity against chlamydiae has been difficult or impossible to induce in any animal model of chlamydial infection. The chronic inflammation resulting from repeated reinfection of humans is believed to have an important role in the blinding sequelae of trachoma (17, 30). The immunologic mechanisms responsible for the relatively poor protection from repeated chlamydial infection are not well understood. The vigorous serologic responses to some chlamydial antigens may occur without sufficient effector T-cell responses to provide adequate protection from reinfection (5–7, 14, 15, 25). Alternatively, the intense inflammatory response to one or more chlamydial antigens may interfere with specific protective immune functions (22, 26, 27). Chlamydiae have been shown under some conditions to be polyclonal stimulators of B or T lymphocytes (2, 10), and if this nonspecific activation occurred in vivo it might also prevent the development of protective immunity.

The biochemical nature of chlamydial elementary bodies (EB) and the relative importance of a variety of EB surface antigens have been investigated by several groups (1, 6, 7, 15, 18, 19). Serologic responses during infection are dominated by those to the major outer membrane protein (MOMP) and lipopolysaccharide of chlamydiae. Recently, Watkins and associates described the isolation of a chlamydial antigen, obtained by Triton X-100 extraction (TE) of viable EB (26). This antigen has not yet been fully characterized, but it has been shown in both the monkey model (22) and the guinea pig model (26) to induce an ocular delayed-type hypersensitivity-like response in previously immunized animals that mimics the clinical disease. Neither MOMP nor killed EB elicited such a response. This delayed-type hypersensitivity-like effect has been proposed to have a role in the pathogenesis of trachoma (22).

The present studies were performed to determine whether a primary infection or the TE-induced inflammatory response within the conjunctivae of ocular immune monkeys was associated with the presence of chlamydia-specific lymphocytes or whether the conjunctival infiltrate was due merely to a nonspecific inflammatory response. We performed in vitro assays with conjunctival lymphocytes obtained by collagenase digestion of tissue biopsies from both TE-challenged and acutely infected monkeys. Our studies demonstrated both antigen-specific proliferative responses to homologous EB and in vitro chlamydia-specific antibody production by freshly isolated conjunctival lymphocytes.

MATERIALS AND METHODS

Animals. Young adult cynomolgus monkeys (*Macaca fasicularis*) were used in these studies (Charles River Primates, Inc., Port Washington, N.Y.). All procedures were performed on anesthetized monkeys (Ketaset) and conformed to National Institutes of Health guidelines for the use of animals in research.

Topical TE challenge and infection of monkeys. (i) Three monkeys that had recovered clinically from previous primary ocular infection with viable *C. trachomatis* B serovar EB (B-EB) (strain TW-5; 2,000 infection-forming units [IFU] per eye), ocular immune monkeys, were topically challenged in each eye with 20 μl of TE antigen (1 mg/ml) (22). Conjunctival biopsies were obtained 24 to 48 h after TE challenge.

(ii) In a second experiment, four ocular immune monkeys were challenged repeatedly by ocular inoculation with TE antigen that had been treated so that the levels of detergent were <0.001% (DTE; 1 mg/ml). After antigen extraction with Triton X-100, the solution was run over an Extracti-Gel D column (Pierce Chemical Co., Rockford, Ill.) to remove excess detergent. Detergent removal was verified by testing for erythrocyte lysis. The clinical responses of these animals were followed for 5 weeks.

(iii) In another series of experiments, two naive monkeys were infected with viable B-EB at the same dosage used for group (i). Conjunctival biopsies were removed for culture 14 to 28 days later.

(iv) A final group of four naive monkeys received three applications of the TE (1 mg/ml) on days −35, −21, and −14 before ocular challenge with viable B-EB (2,000 IFU/ml). The effects of pretreatment with TE on the clinical and microbiologic disease were followed for 14 weeks.

Clinical examination and scoring. Clinical scoring for the

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degree of conjunctival inflammation and the follicular response was described in detail previously (24). All monkeys were examined regularly with a slit lamp without the examiner having knowledge of their treatment. The clinical response was graded on a scale of 0 to 3 for each of 10 signs of conjunctival inflammation to obtain a total inflammatory score for each monkey. The 10 clinical signs graded were as follows: the follicular response in the bulbar, limbal, superior tarsal, superior fornix, and inferior fornix portion of the conjunctiva; hyperemia or injection of the bulbar, superior tarsal, superior fornix, and inferior portion of fornix conjunctiva; and ocular discharge. The scores for both eyes were added together to give a total clinical disease score for each monkey. The mean clinical disease score for each group of monkeys was then determined.

Isolation of CL. Strips of conjunctiva (approximately 4 by 12 mm) were removed from the upper or lower fornix of one eye of each monkey at the peak of the inflammatory response and placed into Hanks balanced salt solution containing streptomycin (100 μg/ml) and gentamicin (10 μg/ml). Tissue was minced and incubated in a 0.02% collagenase (no. 4194; Worthington Diagnostics, Freehold, N.J.) solution in Hanks balanced salt solution at 37°C for 3 h. A fivefold dilution of the enzyme-cell preparation with Hanks balanced salt solution containing 10% fetal calf serum was used to terminate the digestion. The digested tissue-cell suspension was washed twice with Hanks balanced salt solution-fetal calf serum and suspended in culture medium (RPMI 1640 containing 10% fetal calf serum, 3×10^{-5} M 2-mercaptoethanol, 1 mM L-glutamine, streptomycin, and gentamicin) at a concentration of 10^5 cells per ml or greater. A typical cell yield from a moderately inflamed conjunctiva after topical TE was 1×10^6 to 4×10^6 cells. Two to five times more cells were obtained from an acutely infected conjunctiva 7 to 21 days postinoculation. Conjunctival tissue was not collected from uninfected monkeys, since we have shown previously that no B lymphocytes and only a few $CD8^+$ T lymphocytes are present in normal monkey conjunctivae (29). The fibroblast contamination was variable and over the 4-day culture period did not noticeably interfere with lymphocyte function. The majority of cells isolated were lymphocytes, and approximately 50% of the cells were $CD4^+$ or $CD8^+$ T lymphocytes as determined by immunohistochemical staining of cytocentrifuge preparations. The inflammatory and follicular responses had begun to wane 21 to 28 days after infection, and decreasing numbers of T and B lymphocytes were present. Ocular immune monkeys that had recovered from infection were challenged with TE antigen at 12 weeks postinfection; at that time only a few residual conjunctival lymphocytes (CL) were present, and follicles were not seen.

Proliferative assay of lymphocyte function. CL or peripheral blood mononuclear cells (PBMC) were cultured for 4 days in microdilution wells in the presence of antigen or mitogen. PBMC were isolated by the method of Young and Taylor (30). PBMC (10^5 cells per well) or CL (2×10^4 cells per well) were added to round-bottom wells, followed by 100 μl of various concentrations of homologous B-EB or heterologous serovar L_2-EB. We determined the optimal EB concentrations by 10-fold dilutions over a 6- to 8-$\log_{10}$ range. Higher concentrations were either toxic or nonspecifically stimulated normal monkey PBMC. The L_2 serovar was tested at 10^6 to 10^8 IFU/ml and for biohazard reasons was UV inactivated before use. Additional wells received 100 μl of either concanavalin A (ConA; 1 μg per well) or pokeweed mitogen (PWM; 1 μg per well). In some experiments, responses to chlamydial antigens (serovar B MOMP or TE and lipopolysaccharide from serovar L_2) were tested. Control wells received only cells and culture medium or the appropriate diluent for the respective antigen. All assays were performed in triplicate. On day 3, 1 μCi of [^{3}H] thymidine (25 μl) was added to each well for an additional 16 to 20 h of culture, after which wells were harvested onto glass fiber filters by using a multiple automated sample harvester (MASH) and distilled water. Filters were counted in a Beckman liquid scintillation counter, and mean counts per minute were determined for replicate wells. The stimulation index (SI) was determined by the formula (experimental counts per minute)/(medium control counts per minute). An SI of 4.0 or greater was considered positive for PBMC since this level surpasses the range of nonspecific responses by normal monkeys. An SI of 2.0 or greater was considered positive for CL.

In vitro production of antichlamydial antibody. Additional cultures of conjunctival cells were established in which cells were cocultured with PWM or medium alone, but the radiolabel was not added. No antigen was added to any of the cultures, to avoid adsorption of secreted antibody. Instead, culture supernatants were collected at weekly intervals and tested for the presence of antichlamydial antibody (immunoglobulin G [IgG] or IgA) in an enzyme-linked immunosorbent assay (ELISA). Supernatants from 4 to 12 replicate wells were pooled, aliquoted, and stored at −85°C until testing. After removal of 100 μl of supernatant per well, cells were fed with complete culture medium containing recombinant human interleukin-2 (500 U/ml; Boehringer Mannheim Diagnostics, Indianapolis, Ind.), alternating with weekly restimulation with PWM to favor long-term survival of cells in culture.

ELISA for antichlamydial antibody. The ELISA was performed as previously described with purified live B-EB as the antigen (W. M. Berg, J. A. Whittum-Hudson, J. Schachter, and H. R. Taylor, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, E88, p. 123). Plates were coated with approximately 10^6 EB per well overnight at 4°C. Freshly thawed B-EB were diluted in phosphate-buffered saline (PBS; pH 7.2 to 7.4) for use as the antigen. The following day, serial dilutions of culture supernatants diluted in PBS-Tween 20-gelatin were added to each well in duplicate (100 μl per well). Starting dilutions were 1:1 or 1:2. Plates were incubated for 90 min at 37°C. Control wells contained no antigen but were otherwise included in all other steps. After three washes with PBS-Tween, alkaline phosphatase-conjugated anti-human IgG or IgA (Kirkegaard and Perry, Gaithersburg, Md.) diluted in PBS-gelatin was added to each well for an additional 60 min at 37°C. The reaction was developed with nitrophenylphosphate in diethanolamine buffer until the positive control sample reached a predetermined optical density at 405 nm of 2.5 (IgG) or 1.7 (IgA), and the entire plate was read on a Biotek manual ELISA reader. Positive control samples for IgG and IgA were included on each assay plate. The titer of each unknown sample was determined as the last dilution having an optical density at 405 nm greater than 3 standard deviations above the mean optical density for a pool of known negative samples.

Immunoperoxidase staining of conjunctival tissue. The major lymphocyte subsets were identified by avidin-biotin complex immunoperoxidase staining of frozen conjunctival tissue sections (29). Acetone-fixed tissue was blocked with normal goat or horse serum, followed by application of primary antibodies directed against $CD4^+$, $CD8^+$, or the major immunoglobulin classes. Monoclonal mouse anti-

TABLE 1. In vitro CL responses to chlamydiae

In vivo treatment[a]		Monkey no.	Mean cpm of [^{3}H]thymidine incorporated (SI)[b]				
Ocular infection	Topical TE		Mitogen[c]		B-EB (IFU/ml)[d]		Control
			ConA	PWM	4×10^7	4×10^6	
Active (primary)	−	B16	40,805 (76)	30,274 (56)	61,677 (114)	36,822 (68)	539
	−	B17	13,974 (31)	15,020 (34)	70,627 (159)	32,420 (73)	444
Ocular immune (recovered)	+	A6	11,830 (4)	ND[e]	8,851 (3)	7,375 (2)	2,969
	+	A7	17,460 (8)	ND	12,439 (6)	11,108 (5)	2,085
	+	A9	46,084 (18)	ND	56,772 (22)	35,731 (14)	2,603

[a] Conjunctival biopsies were obtained 2 to 4 weeks after primary infection or 24 h after topical challenge of ocular immune monkeys with TE extracted from B-EB.
[b] Tissues were collagenase-digested to obtain single-cell suspensions of mononuclear cells (2×10^4 to 5×10^4 cells per 0.1 ml), which were cultured for 4 days in microdilution plates with an equal volume of stimulant or medium. Then 1 μCi of [^{3}H]thymidine was added for the last 16 to 20 h of culture. Results are representative of more than three independent experiments with acutely infected and TE-challenged ocular immune monkeys.
[c] Final mitogen concentrations were 5 μg/ml.
[d] These EB concentrations did not stimulate PBMC from normal monkeys; higher concentrations were often inhibitory in proliferative assays.
[e] ND, Not determined.

human antibodies were used to identify the T-cell subsets (OKT4A and OKT8F, 1:50; Ortho Diagnostics, Inc., Raritan, N.J.). Polyclonal anti-human immunoglobulin antibodies (IgG, IgM, or IgA; 1:100) were used to identify B cells. After incubation at room temperature and washes in PBS, slides were incubated with biotinylated secondary antibodies (1:200; Vector Labs, Burlingame, Calif.) for 30 min. After additional washes, the avidin-biotin complex reagents were applied for 45 min, followed by development of a red reaction product with aminoethylcarbazole in dimethyl sulfoxide containing 0.01% hydrogen peroxide. Slides were counterstained with hematoxylin and mounted with Gelvatol.

RESULTS

Antigen-specific proliferative responses by conjunctival lymphocytes. Conjunctival lymphocytes isolated from inflamed conjunctivae after challenge with TE or infectious B-EB were cocultured with viable whole chlamydiae, chlamydial antigens, or nonspecific mitogens. Dose-dependent responses to B-EB were observed for three of five monkeys. The remaining two monkeys showed a similar positive response (SI, >2) at the two concentrations of EB (Table 1). The SIs at the optimal B-EB concentration of 4×10^7 IFU/ml ranged from 3 to 22 for three monkeys challenged with TE. These were somewhat lower than the proliferative responses elicited from CL obtained during primary infection (SIs, 66 to 159). The SIs of TE-induced conjunctival lymphocytes to ConA ranged from 4 to 18 in this experiment and were also lower than the responses of CL from infected monkeys (SIs, 31 to 77). These differences may have been present because background [^{3}H]thymidine incorporation was higher for CL isolated after TE challenge, perhaps because of some unidentified stimulatory effects of the TE antigen. Background responses by conjunctival cells in other experiments varied from 250 to 4,000 cpm. PBMC collected at the same times as conjunctival tissue were stimulated in parallel cultures, and dose-dependent responses to B-EB were observed for all five monkeys (Table 2). The PBMC from the monkey that exhibited the highest conjunctival lymphocyte responses (A9) also responded most vigorously to all stimulating agents (compare Tables 1 and 2).

Proliferative responses to heterologous UV-inactivated L_2-EB (10^6 to 10^8 IFU/ml) by either CL or PBMC were not observed in these experiments (SI, <1.0), regardless of in vivo treatment. Responses to high-pressure liquid chromatography-purified MOMP and the TE antigen were tested over broad concentration ranges of 1:50 to 1:100,000 and were negative (SI, <2.0). The control cultures were stimulated with the diluent used to suspend these antigens. This lack of responsiveness may have been due to the toxic effect of the detergents used to originally extract the proteins; once

TABLE 2. In vitro PBMC responses to chlamydiae

In vivo treatment[a]		Monkey no.	Mean cpm of [^{3}H]thymidine incorporated (SI)[b]				
Ocular infection	Topical TE		Mitogen[c]		B-EB (IFU/ml)[d]		Control
			ConA	PWM	4×10^7	4×10^6	
Active	−	B16	108,604 (129)	43,901 (52)	10,674 (13)	4,975 (6)	840
	−	B17	115,143 (195)	59,923 (101)	17,603 (30)	4,918 (8)	591
Ocular immune (recovered)	+	A6	21,837 (95)	8,844 (38)	1,350 (6)	532 (2)	213
	+	A7	68,124 (238)	8,830 (30)	4,957 (17)	539 (2)	286
	+	A9	161,606 (284)	36,107 (63)	46,429 (81)	9,694 (17)	570

[a] PBMC were obtained at the same times as conjunctival biopsies (Table 1).
[b] Single-cell suspensions of PBMC (10^5 cells per 0.1 ml) were cultured for 4 days in microdilution plates with an equal volume of stimulant or medium. Then 1 μCi of [^{3}H]thymidine was added for the last 16 to 20 h of culture. Results are from triplicate cultures and are representative of more than three independent experiments.
[c] Final concentrations of mitogens were 5 μg/ml.
[d] These EB concentrations were optional and did not induce responses by PBMC from normal monkeys. SIs at 4×10^5 IFU/ml were all <4.0.

TABLE 3. Antichlamydial antibody produced in vitro by CL

Monkey	Days in vitro[a]	Antichlamydial ELISA titer[b]			
		PWM stimulated		Control	
		IgG	IgA	IgG	IgA
A6	7	<1:2	1:32	<1:2	1:2
	21	1:2	1:8	1:2	1:2
A7	7	1:2	1:2	1:8	1:2
	21	1:2	<1:2	1:4	1:2
A9	7	1:32	1:16	1:32	1:8
	21	>1:32	1:16	1:8	1:4

[a] All cultures (10^4 cells per well) were initiated on the same day; supernatants (100 μl) were collected and replicates were pooled at days 7 and 21 of in vitro culture with or without PWM. Cells were fed on alternate weeks with medium or medium plus PWM.

[b] All samples from each time point were tested in ELISAs at the same time; viable B-EB (1:1,000; ~10^6 EB per well) were used as the antigen. Plasma samples obtained from these monkeys at the time of conjunctival biopsy indicated antichlamydial IgG titers of approximately 1:200. However, PBMC (10^5 per well) did not secrete detectable antichlamydial antibody in vitro. Similar results were obtained with cells obtained from actively infected conjunctivae.

the reagents were diluted past toxic levels, the protein concentrations may have been too low to elicit responses. Alternatively, the lack of responses to MOMP or TE may have been due to inappropriate target antigens, considering that the monkeys were initially infected with whole organisms. PBMC responses to UV-inactivated B-EB were similar to responses to viable B-EB. L_2-EB were inactivated because this chlamydial serovar can infect lymphocytes, whereas other trachoma serovars infect only epithelial cells (13).

Chlamydia-specific in vitro antibody production by CL. Additional microcultures of CL were established at the same time as proliferative assays. These cultures were stimulated with PWM but without thymidine labeling. Culture supernatants were collected and tested by ELISA for the presence of chlamydia-specific antibody after 7 to 51 days of in vitro culture. Supernatants from 7- and 21-day cultures of TE-induced CL contained chlamydia-specific antibody at titers of 1:2 to 1:32 (Table 3). Cultured CL from two TE-challenged monkeys contained B cells producing detectable levels of antichlamydial IgG antibody as late as 31 to 51 days after in vitro culture (data not shown). Importantly, stimulation with PWM was not required for in vitro antibody production during the first 7 days of culture, although PWM enhanced long-term cell survival and appeared to enhance antibody secretion by cells from some monkeys. CL for monkey A6, which had exhibited the lowest proliferative responses to all stimulants (Tables 1 and 2), produced more IgA than IgG throughout this experiment (Table 3). We failed to detect antibody in supernatants from cultured PBMC from the same monkeys even when cultures contained 10- to 20-fold more cells (data not shown). Control culture medium (no cells) was also negative. PWM-stimulated CL obtained during an active ocular chlamydial infection produced levels of antichlamydial antibody similar to those observed after noninfectious TE challenge (data not shown). These results demonstrate that chlamydia-specific B lymphocytes are present in the conjunctiva during ocular chlamydial infection.

Clinical effects of ocular challenge with chlamydial antigen extract. Our previous studies have shown that the conjunctiva becomes progressively inflamed and infiltrated with T lymphocytes over the first 2 weeks after primary ocular chlamydial infection. Follicles appear and persist over the next 8 to 12 weeks, whereas inflammation gradually declines (29). In the present study, the eyes of four normal monkeys remained clinically normal through three separate applications of TE antigen given 1 or 2 weeks apart. TE antigen applied before ocular infection had no effect on clinical disease scores (Fig. 1) or recovery of viable organisms (data not shown) upon challenge with live B-EB 1 week after the last TE application. These results demonstrate that this chlamydial extract antigen did not induce either sensitization or protection in naive monkeys.

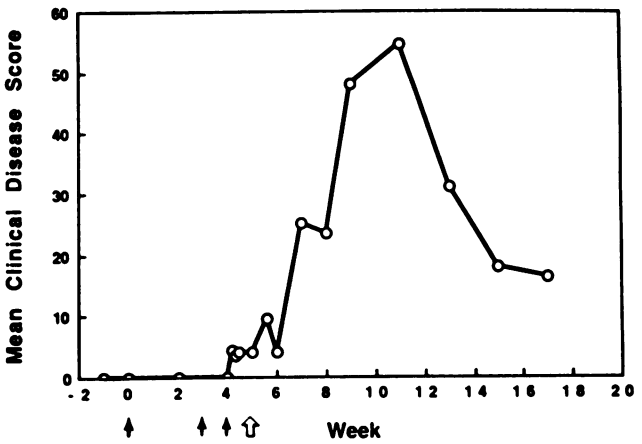

FIG. 1. Effect of topical TE on the responses of four naive monkeys to ocular infection with B serovar (2,000 IFU per eye). TE failed to induce ocular inflammation or follicles as evidenced by the clinical disease score before ocular infection. After infectious challenge, the clinical response was similar to that of naive monkeys, as was the ability to recover viable organisms (data not shown). TE was applied three times (closed arrows) before ocular infection on day 35 (open arrow).

Monkeys that had recovered from a previous ocular chlamydial infection with serovar B (ocular immune animals) were treated topically with DTE prepared from B-EB.

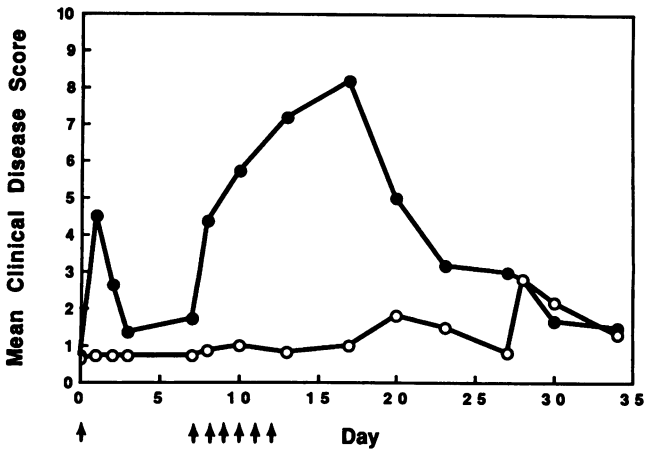

FIG. 2. Clinical disease scores after topical administration of DTE to eyes of four ocular immune monkeys. Monkeys had recovered from a previous chlamydial ocular infection with B serovar (TW-5), and their eyes were then topically challenged with DTE (○) or PBS (●). The clinical disease score is derived from the inflammatory and follicular indices as described in the text. Arrows indicate times of topical administration.

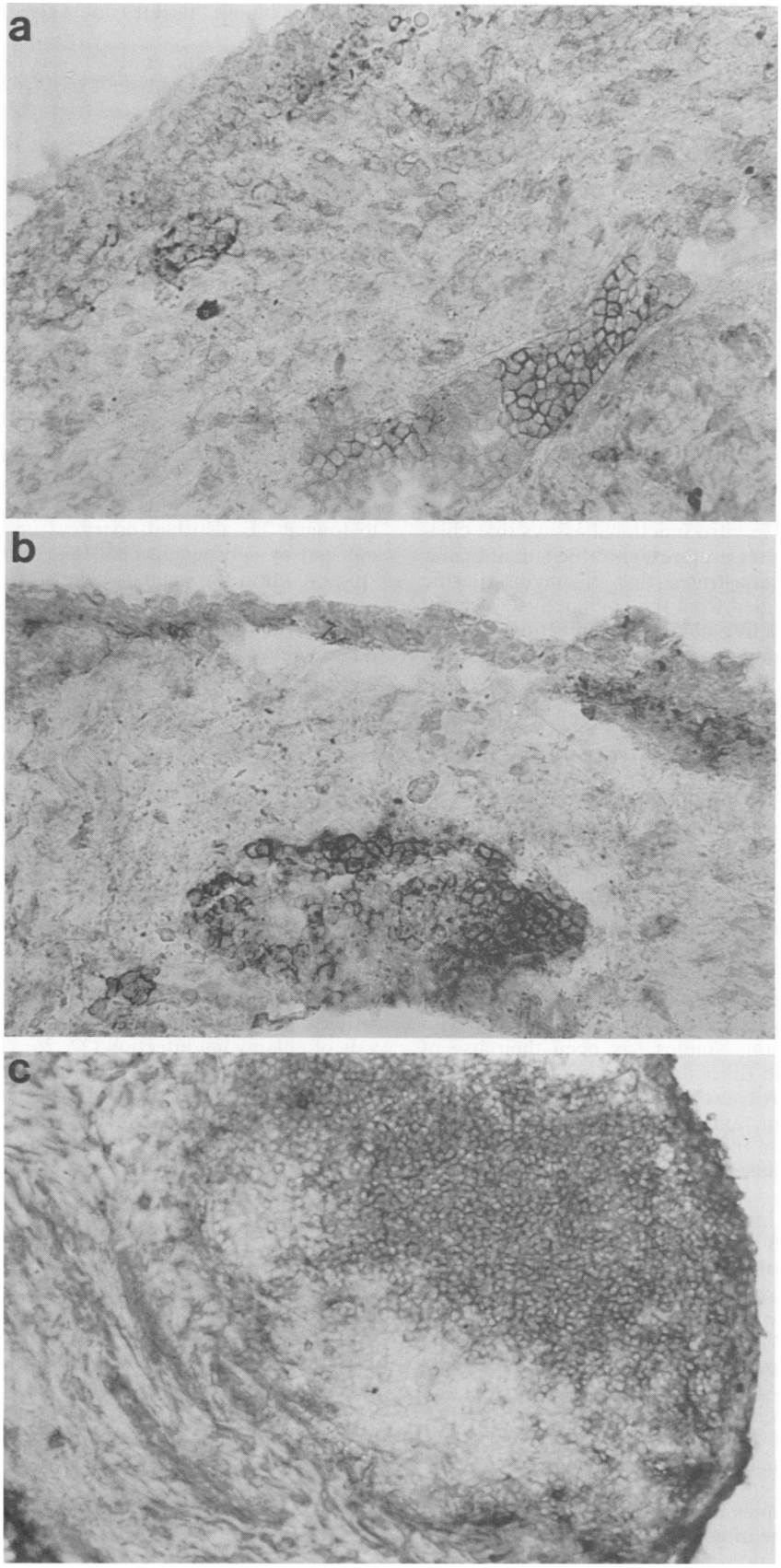

FIG. 3. Immunohistochemical evidence of $CD4^+$ and $CD8^+$ infiltration and follicle formation after topical TE challenge of immune monkeys. Serial frozen sections were stained by the avidin-biotin complex immunoperoxidase technique for (a) $CD4^+$ cells, (b) $CD8^+$ cells, and (c) IgG-bearing B cells. Magnifications: a and b, ×100; c, ×63.

Topical DTE induced significant clinical disease scores in immune animals, including the appearance of ocular disease clinically characterized by the presence of an inflammatory infiltrate and follicles (Fig. 2). Histopathologically, abundant $CD8^+$ cells and fewer $CD4^+$ cells were observed 24 to 48 h after topical DTE treatment (Fig. 3). The clinical disease score decreased rapidly to background levels over the next 2 to 3 days. When DTE applications were resumed on a daily basis, a marked and sustained inflammatory and follicular response was observed. Inflammation continued to increase for 1 week upon cessation of DTE treatment and then slowly waned over the next 2 weeks (Fig. 2). Topical application of PBS produced no clinical disease in eyes of ocular immune animals.

DISCUSSION

Although follicle formation and T-cell infiltration of chlamydia-infected conjunctivae have been well documented (17, 20, 23, 28, 29), the antigen specificity of these lymphocytes has not been known. These studies are the first to directly demonstrate by in vitro functional assays that chlamydia-specific lymphocytes are present at the site of ocular infection. We have demonstrated that chlamydia-specific lymphocytes can be isolated from the conjunctivae of monkeys either during an active chlamydial infection or after topical challenge of immune monkeys with TE from the homologous B serovar of *C. trachomatis*. Proliferative responses to both homologous EB and to the T-cell mitogens ConA and PWM were observed. The in vitro proliferative responses to chlamydiae occurred at concentrations that did not stimulate nonspecific proliferation of normal monkey PBMC. In addition, in vitro production of chlamydia-specific antibody by conjunctival B cells was demonstrated as early as 7 days after culture and for at least 7 weeks for one monkey.

Chlamydiae have been demonstrated to induce proliferation by T lymphocytes (4, 8, 16, 31) and B lymphocytes (2, 10, 11) in several systems ranging from human PBMC to mouse spleen cells. In some cases, in vitro proliferative responses have been regarded as due to nonspecific polyclonal stimulation of B cells (2, 10). A potential problem with interpreting these previous studies has been their use of different host species and EB of different serovars, including *C. trachomatis* serovar L_2, the biovar that causes lymphogranuloma venereum, and various *Chlamydia psittaci* strains. Our studies have utilized a human ocular strain of chlamydiae known to cause trachoma in the primate model (21, 23).

Although our studies have not defined whether the proliferative responses to EB were limited to one or more lymphocyte subsets, the positive responses to ConA and PWM established that functional T cells were indeed present in the conjunctival lymphocyte preparations. The lack of in vitro responses to MOMP or TE antigen may have been simply a concentration problem at nontoxic dilutions and will require further investigation. Work by Young and Taylor (31) and others (11; L. E. Guagliardi, G. I. Byrne, and D. M. Paulnock, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, D164, p. 93) has shown the delayed appearance of $CD8^+$ peripheral blood or spleen cells that suppress responses to L_2-EB and to ConA. James and co-workers (9) found nonspecific $CD8^+$ cells in the intestinal mucosa after serovar L_2 rectal infection. In the present studies, both the actively infected and TE-challenged monkeys had effective ConA responses at the time of collection of conjunctivae and peripheral blood; this does not suggest the presence of active suppression.

Chlamydia-specific antibody was detectable after in vitro culture of conjunctival lymphocytes but not of 10-fold higher numbers of PBMC collected from the same monkeys at the same times. This suggests that the frequency of antigen-specific B cells is much higher in conjunctivae than in peripheral blood.

The precise mechanism by which TE elicits such a rapid inflammatory response in the conjunctivae of sensitized or ocular immune animals remains to be determined. However, a 57-kilodalton protein has been recently purified and shown to elicit inflammatory responses in guinea pigs similar to those induced in monkeys by the cruder TE (12). The 57-kilodalton protein has now been sequenced and shown to have homology with procaryotic stress proteins (R. Morrison, K. Lyng, and R. J. Belland, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, B24, p. 34).

Repeated inoculation of TE induced no clinical disease when administered to naive monkeys that had never been infected with chlamydiae. Prior exposure of normal animals to topical TE also had no effect upon the clinical disease induced by subsequent challenge with live organisms. Our studies with naive animals have shown that TE itself is not immunogenic when applied topically, as it induces neither hypersensitivity nor protection. This is not due to inadequate delivery or penetration of antigen, because the same TE dose produces a marked inflammatory response in immune animals. The induction of chronic persistent disease in ocular immune animals with the DTE antigenic preparations is noteworthy. It provides a noninfectious model for chronic trachoma that may prove particularly useful for the study of the immunopathogenesis of this disease.

It will be important to determine whether one or more of the T-cell subpopulations which appear in the TE-challenged conjunctiva act directly to increase inflammation by specific responses to antigen or indirectly via lymphokine production by mononuclear cells during the inflammatory response. Antigen-specific conjunctival lymphocytes were found in both TE-induced inflammation and actively infected conjunctivae. Our results support the hypothesis that hypersensitivity to chlamydial antigen(s) has a role in the pathogenesis of chlamydial infection (22, 26). The primate models of acute and chronic ocular chlamydial infection should be of great value in addressing these questions of conjunctival immune responsiveness and should have broader application to chlamydial infections at other mucosal sites.

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