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An In Vitro Model for Immune Control of Chlamydial Growth in Polarized Epithelial Cells

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A polarized epithelial culture system and chlamydia-specific T-cell lines and clones were employed to investigate the ability and mechanisms by which T cells control the growth of chlamydiae in epithelial cells. Monolayers of polarized mouse epithelial cells were infected with the *Chlamydia trachomatis* agent of mouse pneumonitis (MoPn) and then exposed to antigen-stimulated MoPn-specific T-cell lines and clones. The results revealed that in vivo-protective MoPn-specific T-cell lines and clone 2.14-0 were capable of inhibiting the growth of MoPn in polarized epithelial cells. In contrast, the nonprotective MoPn-specific T-cell clone 2.14-3, naive splenic T cells, and a control T-cell clone could not inhibit the growth of MoPn in epithelial cells. Transmission electron microscopic analysis of infected epithelial cells which were exposed to clone 2.14-0 confirmed the absence of an established infection, as deduced from the virtual absence of inclusions in the cells. Antigen-specific activation of clone 2.14-0 was required for the MoPn-inhibitory function, since the absence of antigenic stimulation or stimulation with a heterologous chlamydial agent did not result in MoPn growth inhibition. Activation of clone 2.14-0 resulted in acquisition of the capacity to inhibit growth of both homologous (MoPn) and heterologous chlamydial agents. Close interaction between epithelial cells and clone 2.14-0 was required for the MoPn-inhibitory action, because separation of the cell types by a filter with a pore size of 0.45, 3.0, or even 8.0 μm abrogated MoPn inhibition. Protective T cells may act at close range in the epithelium to control chlamydial growth, possibly involving short-range-acting cytokines. The ability of antigen-stimulated T-cell lines and clones to inhibit chlamydial growth in polarized epithelial cultures could be a useful method for identifying protective T-cell clones and antigenic peptide fragments containing protective epitopes.

Chlamydial infections are widespread as both the most common sexually transmitted disease in the United States and the cause of trachoma, the world's leading cause of preventable blindness (36). Pelvic inflammatory disease is a major complication of chlamydial genital disease in women and may lead to fallopian tube damage and infertility (32).

The genital or ocular mucosal epithelium is the target of chlamydial infection, and specific infection-driven mononuclear cell infiltration and lymphoepithelial interactions are associated with the exposure (22, 23, 38). These interactions do lead to the elicitation of antichlamydial immune responses, with resultant development of specific immunity and resolution of disease (6, 11, 13, 27, 28). Humoral immunity as well as cell-mediated immunity have been found to have protective functions in chlamydial genital disease (12, 21, 25–27, 31). Paradoxically, T-cell-mediated antichlamydial immune responses, possibly directed at specific chlamydial antigens on the epithelium during recurrent and/or persistent infections, are also responsible for the pathogenesis of conjunctival scarring in trachoma (7, 19, 37) and possibly pelvic inflammatory disease-associated fallopian tube damage as well (40).

We have been studying the T-cell immune mechanisms involved in the resolution of chlamydial genital infection in the murine model of the disease. In this system, when immunocompetent +/*nu* mice ($H\text{-}2^d$) are infected intravaginally with the *Chlamydia trachomatis* biovar agent of mouse pneumonitis (MoPn), the infection is resolved within 3 weeks. T-cell immunity alone in the absence of humoral immunity is capable of bringing about the resolution of chlamydial genital infection in susceptible mice (8, 9, 24, 25, 30). Thus, B-cell-deficient mice could resolve the disease as efficiently as immunocompetent mice (25). Also, athymic *nu/nu* mice, which are normally incapable of resolving the infection (30), can be cured of the disease by adoptive transfer of MoPn-specific T-cell lines (24) or clones (8, 9).

Studies with in vitro models consisting of polarized epithelial (and nonepithelial) cell cultures have indicated that a major mechanism of chlamydial colonization of the epithelium is receptor-mediated endocytic internalization in clathrin-coated pits (46). However, no information is currently available on the nature or types of lymphoepithelial interactions or their effects on chlamydial colonization of the epithelium and the intracellular growth of the pathogen or how such interactions lead to protective immunity or immunopathology of chlamydial disease. Knowledge of the cellular and molecular mechanisms of genital or ocular lymphoepithelial interactions during chlamydial infection and their effects on chlamydial colonization, multiplication, and spread in the genital or ocular epithelium is important for present efforts to design and develop vaccines against the disease (4).

To understand the cellular and molecular mechanisms of T-cell restriction of chlamydial growth in the epithelium and the immunopathogenic mechanisms of epithelial injury by antichlamydial cell-mediated immunity, we have begun a systematic investigation of the effect of lymphoepithelial interactions on chlamydial infection by using a polarized epithelial-lymphocyte coculture (PELC) system. These studies involve

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the establishment of cultures of polarized mouse epithelial cells, infection with MoPn, and exposure of infected cells to MoPn-specific T-cell lines and clones. The T-cell lines and clones have been characterized as either protective or nonprotective on the basis of their ability to cure genital MoPn infection in nude mice (8, 9, 24). The PELC system is especially suitable for these studies because under normal physiologic conditions epithelial cells are polarized in their organization, and the interaction with immune cells results in control of chlamydial infection. The present report describes the adapted PELC system and its use for examining the effect of T cells on the productive growth of MoPn in epithelial cells.

MATERIALS AND METHODS

Animals. Female +/*nu* mice on a BALB/c background (*H-2*^{d}), 5 to 6 weeks old, were obtained from Harlan Sprague-Dawley, Inc., Indianapolis, Ind., and maintained in Plexiglas isolators under pathogen-free conditions. The animals were fed with food and water ad libitum under 12-h-light and 12-h-dark conditions.

Cultivation of chlamydiae and preparation of antigens. Chlamydial stocks used for infecting polarized epithelial monolayers were prepared according to standard techniques using HeLa cells, as previously described (25). Antigens from MoPn, *C. trachomatis* serovar E, and the *Chlamydia psittaci* agent of guinea pig inclusion conjunctivitis (GPIC) were prepared by purification of elementary bodies (EBs) from stocks with Renografin gradients (25) and exposing the EBs to UV light for 3 h.

Epithelial cells and polarized epithelial cell culture. The epithelial cell line employed for these studies, TM3 (ATCC CRL 1714), was derived from Leydig cells of BALB/c +/*nu* mice (*H-2*^{d}). The cells have normal or primary epithelial cell culture characteristics (17) and are nontumorigenic. They are responsive to luteinizing hormone (by an increase in cyclic AMP secretion) but are nonresponsive to follicle-stimulating hormone. TM3 cells express receptors for estrogen, epidermal growth factor, androgen, and progesterone and secrete prostaglandin F_2. The cell line was adapted to and maintained in culture in RPMI 1640 complete medium, composed of RPMI 1640 (Hazelton Research Products, Denver, Pa.) supplemented with 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (GIBCO Laboratories, Grand Island, N.Y.), 10% heat-inactivated fetal bovine serum (GIBCO), 1.0 mM sodium pyruvate, 0.1 mM nonessential amino acids, 2 mM glutamine, 2×10^{-5} mM 2-mercaptoethanol (Sigma Chemical Co., St. Louis, Mo.), 50 μg of gentamicin per ml or 100 U of penicillin per ml (GIBCO), and 100 μg of streptomycin per ml (GIBCO). We have established that chlamydial agents employed in these studies (MoPn, GPIC, and *C. trachomatis* serovar E) grow efficiently in TM3 cells in culture.

To establish monolayers of polarized epithelial cell cultures that mimic the in vivo architecture of the mucosal epithelium, 1×10^6 to 2×10^6 TM3 cells were seeded into the inner chamber of multi-well Transwell plates (24-mm diameter, collagen-coated polycarbonate filter; pore size, 0.45 μm; catalog no. 2435; Costar, Cambridge, Mass.) according to the method of Wyrick and coworkers (46). The polarized monolayer is attained after 24 to 48 h of culture at 37°C. Centrifugation did not affect the polarization of the cells as determined by inverted microscopic observation.

Infection of polarized epithelial cell cultures. Unless otherwise stated, infection of polarized epithelial monolayers was carried out with 10^3 inclusion-forming units (IFU) of MoPn or GPIC in a volume of 0.25 ml of the growth medium. The outer chamber contained 2 ml of the growth medium. Infected epithelial cells were centrifuged at 2,060 × *g* for 30 min.

TABLE 1. Properties of T-cell preparations employed in the studies

T cells	Phenotype	Antigen specificity	Ability to resolve chlamydial infection[a]
Clone 2.14-0	$CD4^+$	MoPn	Yes
Clone 2.14-3	$CD4^+$	MoPn	No
Clone TC08AA-5	$CD4^+$	Allospecific	No
T-cell lines (JR1 and JR2)	$CD4^+$ $CD8^+$	MoPn	Yes
NSTC[b]	$CD4^+$ $CD8^+$	None	No

[a] Resolution of disease was established by at least three consecutive negative isolation attempts from cervicovaginal swabs.
[b] NSTC, normal splenic T cells.

Production of MoPn-specific T cells and clones. Table 1 summarizes the general characteristics of the T-cell preparations employed in these studies. The isolation procedure and properties of the three T cell lines and clones employed in these studies have been previously described (8). Briefly, clones 2.14-0 and 2.14-3 are both MoPn-specific, $CD4^+$ T cells that were generated by the limiting dilution technique from splenic cells of MoPn-infected +/*nu* mice. Both clones are antigen specific, major histocompatibility complex restricted, and biovar specific, since they do not respond to stimulation by *C. trachomatis* serovar E or GPIC. While cultures of antigen-stimulated 2.14-0 contained interleukin-2 and high levels of gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α), identical cultures of clone 2.14-3 contained relatively lower levels of IFN-γ (at least fivefold less) and nondetectable levels of TNF-α. In addition, when 10^7 cells of each clone were adoptively transferred into nude mice with established genital MoPn infection, only clone 2.14-0 was capable of curing the recipients.

Clone TC08AA-5 is an allospecific $CD4^+$ clone, employed as an irrelevant control in the studies. It was generated by stimulating +/*nu* splenic T cells with C57BL/6 splenic stimulator cells in a one-way mixed-leukocyte culture and cloned by the limiting dilution method (8, 9).

Other T-cell preparations employed in these studies include short-term MoPn-responsive T-cell lines (JR-1 and JR-2) generated by restimulating and expanding MoPn-immune T cells with MoPn, antigen-presenting cells (APCs), and concanavalin A-conditioned medium as previously described (8) and normal splenic T cells prepared by combined nylon-wool and glass bead purification of spleen cells from naive +/*nu* mice, which yielded at least 99% $CD3^+$ T cells as analyzed by fluorescence-activated cell sorting (24).

Exposure of chlamydia-infected polarized epithelial cells to T cells. All T-cell lines and clones were harvested and purified over Ficoll gradients in order to enrich for viable cells before they were used in these experiments. T cells (2.5×10^6 per well) were stimulated for 24 to 48 h in six-well tissue culture plates with 20×10^6 APCs and 10 μg of antigen per ml in a final volume of 2.0 ml. APCs were prepared by irradiating spleen cells from naive +/*nu* mice with 2,000 rads of X irradiation followed by subsequent washing in HEPES-buffered RPMI 1640 medium. Exposure of infected epithelial cells to activated T cells was carried out by two general methods. (i) The Transwell tissue culture inserts containing infected epithelial cells were transferred into culture wells containing stimulated T cells, in which case the infected epithelial cells were suspended in the culture supernatants of the T cells. In this method, the activated T cells and the infected epithelial

cells were physically separated by a filter with a pore size of 0.45 μm. (ii) The stimulated T cells (with or without the supernatants) were transferred into the Transwell inserts containing infected epithelial cells (coculturing). In this case, the T cells and the infected epithelial cells were physically in contact with one another. T-cell-exposed cultures and controls were incubated for 48 h.

Assessment of chlamydial growth in polarized epithelial cells. At the end of the 48-h incubation period, the supernatants were aspirated from the infected epithelial cells and the filters were carefully cut out with sterile surgical blades, placed in double-sucrose phosphate medium, and stored at −70°C. The productive growth of chlamydiae (MoPn or GPIC) in polarized epithelial cells in the presence or absence of T cells was determined by isolation of the organism from the filter samples in McCoy cells and detection of chlamydial inclusions by microimmunofluorescence assay (25). Each set of isolation experiments was repeated at least three times in order to obtain a quantifiable and consistent pattern of results.

TEM studies. Transmission electron microscopic (TEM) studies were conducted with infected epithelial samples in order to determine the nature of the chlamydial inclusions when MoPn-infected epithelial cells were exposed to chlamydia-specific T cells. Following exposure of MoPn-infected polarized epithelial cells to specific T-cell clones and lines for 48 h, the filters containing the infected epithelial cells were washed and fixed in situ with 2% glutaraldehyde–0.5% paraformaldehyde in 0.1 M cacodylate buffer for 45 min at 37°C. Fixed filter samples were cut out and dipped in 2% molten Noble agar and processed in Epon 812 resin for TEM as previously described (46).

Statistical analysis. The degrees of inhibition of MoPn by different preparations and various numbers of T cells were compared by performing a one-tailed t test, with minimal statistical significance judged at $P < 0.05$.

RESULTS

Effect of MoPn-specific T cells on productive growth of MoPn in polarized epithelial cells. We employed a PELC system to investigate the nature and mechanism of lymphoepithelial interactions that culminate in T-cell immune responses against chlamydiae in the mucosal epithelium. In order to establish that specific T-cell-epithelial cell interactions could control chlamydial colonization of the epithelium, the initial studies were designed to test the effect of exposing chlamydia-infected polarized epithelial cells to antigen-stimulated T cells on the productive growth of chlamydiae. The characteristics and sources of T cells employed in these studies, as given in Table 1, are as follows: (i) MoPn-specific T-cell clones that were either capable of curing established genital MoPn disease in nude mice when adoptively transferred into diseased mice (protective T-cell clones) or were incapable of curing the genital infection (nonprotective T-cell clones); (ii) MoPn-reactive T-cell lines (protective); (iii) an irrelevant allospecific control T-cell clone (nonprotective); and (iv) nylon-wool-purified naive splenic T cells (nonprotective).

To determine whether chlamydia-specific T cells could influence the multiplication of chlamydiae in epithelial cells, replicate cultures of polarized TM3 epithelial cells were established in Costar's Transwell plates and infected with 10^3 IFU of MoPn. Within 30 min of infection, the epithelial cells were cocultured with antigen-stimulated MoPn-specific T-cell clones and other T-cell preparations for 48 h. Transwell filters containing infected epithelial cells were harvested at the end of the incubation period. The effect of the T-cell exposure on the productive growth of MoPn in polarized epithelial cells was determined by isolation of MoPn from the harvested filters and detection of the inclusion bodies by the microimmunofluorescence technique. Figure 1 shows that when protective and nonprotective T-cell preparations were cocultured with MoPn-infected polarized epithelial cells, the protective T-cell lines (JR-1 and JR-2) and clone 2.14-0 could limit the productive growth of MoPn in epithelial cells. Nonprotective T-cell clone 2.14-3 and the control allospecific T-cell clone (TC08AA-5) or naive splenic T cells could not inhibit the growth of MoPn in polarized epithelial cells. These data were substantiated by exposure of MoPn-infected TM3 cells to three other nonprotective MoPn-reactive T-cell clones of both CD4 and CD8 phenotypes; again, no inhibition of MoPn multiplication occurred (data not shown).

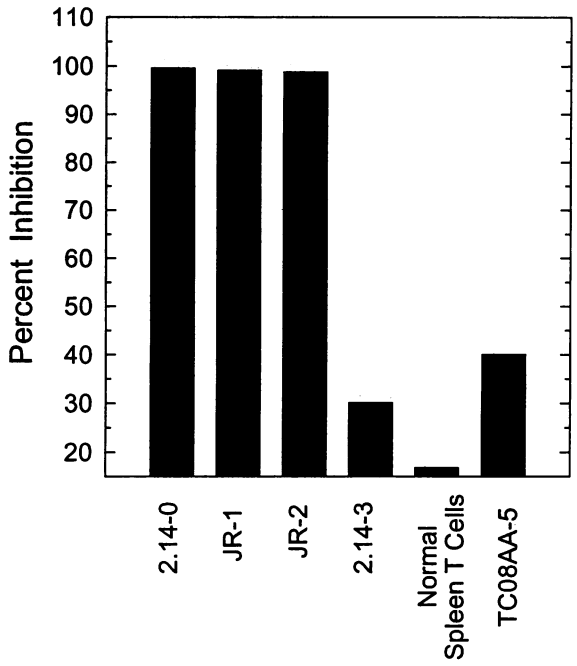

FIG. 1. Effect of exposing MoPn-infected polarized epithelial cells to MoPn-specific T-cell lines and clones. MoPn-infected polarized epithelial cells were cocultured with each T-cell preparation (2.5×10^6 per well) in duplicate wells of Transwell plates. T-cell-exposed cultures and controls were incubated for 48 h, and MoPn growth was assessed by tissue culture isolation and detection of inclusion bodies by the microimmunofluorescence method. Results are expressed as percent inhibition calculated as follows:

$$\frac{\text{mean IFU/ml of control cultures (no T cells)} - \text{mean IFU/ml of experimental cultures (+T cells)}}{\text{mean IFU/ml of control cultures (no T cells)}} \times 100$$

We also investigated whether the ability of clone 2.14-0 to inhibit the growth of MoPn in polarized epithelial cells was dependent upon the stage of the infection, such as the length of time after infection before the epithelial cells were exposed to T cells. This was done because the infected epithelial cells were normally cocultured with T cells within 30 min of infection. To address whether the MoPn-inhibitory action of clone 2.14-0 was dependent upon the stage of the infection, the coculturing of infected epithelial cultures with clone 2.14-0 was delayed for 24 h after the infection. When the growth of MoPn inclusions was determined, it was observed that the delay of 24

h before the exposure of infected epithelial cells to protective T-cell clone 2.14-0 did not affect the ability of the clone to limit the productive growth of MoPn in the polarized epithelial cells (data not shown).

These results indicated that chlamydial multiplication in epithelial cells can be inhibited by chlamydia-specific T cells in vitro and the inhibition is effective even when the infectious process has initiated in the epithelial cells. Further, it appears that only in vivo protective T cells could inhibit chlamydial growth in polarized epithelial cells in vitro, suggesting that a common mechanism of antichlamydial action may be involved.

We additionally wanted to compare the nature of chlamydial inclusions formed in polarized epithelial cells in the presence or absence of protective and nonprotective T cells. In such investigations, TEM studies were conducted on infected epithelial cell cultures that were either exposed or not exposed to protective and nonprotective T cells. Figure 2 represents the transmission electron photomicrographs of MoPn-infected polarized epithelial cell cultures, which were exposed to culture medium alone (Fig. 2A and B, enlarged inclusion) or to the protective clone 2.14-0 (Fig. 2C) and the irrelevant T-cell clone TC08AA-5 (Fig. 2D). MoPn inclusion bodies are detectable in infected epithelial cells that were exposed to medium alone (Fig. 2A and B) or cocultured with nonprotective clone TC08AA-5 (Fig. 2D). In contrast, no inclusions are detectable in infected epithelial cells that were cocultured with the protective clone 2.14-0 (Fig. 2C). The result indicated that protective T cells may control chlamydial disease by limiting the development of inclusions in the infected cells.

Effects of T-cell numbers and MoPn inocula on the ability of clone 2.14-0 to inhibit MoPn growth in polarized epithelial cells. We investigated whether the intensity of the local antigen-specific T-cell response and microbial load could influence the ability of T cells to control epithelial colonization by chlamydiae. In these studies, the effects of varying MoPn inocula with a constant number of cells of the protective T-cell clone 2.14-0 and varying numbers of T cells with a fixed MoPn inoculum were investigated in the PELC system. Replicate cultures of polarized epithelial cells were established in Transwell plates. One set of cultures was infected with various inocula of MoPn (from 10^5 to 10^1 IFU per well) and exposed to a fixed number of clone 2.14-0 cells (2.5×10^6 per well) for 48 h. Another set of polarized epithelial cell cultures was infected with a constant inoculum of MoPn (10^3 IFU per well) and then exposed to various numbers of clone 2.14-0 cells (10^7 to 10^3 per well) for 48 h. The choice of 10^3 IFU per well of MoPn inoculum in the latter set of cultures was based on preliminary experiments which showed that when this dose was employed to infect TM3 epithelial cells with exposure to 2.5×10^6 cells of the protective T-cell clone 2.14-0, a reduction of approximately 2 logs of MoPn IFU over control cultures was observed. The results revealed that the ability of clone 2.14-0 to inhibit the productive growth of MoPn in polarized epithelial cells was dependent upon the number of clone 2.14-0 cells in culture and the MoPn inocula employed. Thus, when the number of clone 2.14-0 cells was increased in cultures relative to a fixed inoculum of MoPn, the ability of the clone to inhibit the productive growth of MoPn in epithelial cells was also increased (Fig. 3). Extremely low numbers of clone 2.14-0, such as 10^3 or 10^4, had no significant inhibitory effect on the growth of MoPn in epithelial cells as determined by the one-tailed *t* test. Conversely, when the MoPn inoculum was steadily increased, the ability of a fixed T-cell population to control the multiplication of the organism in epithelial cells was reduced at 10^5 IFU (Fig. 4) ($P < 0.0001$ for all groups when compared with the control). The results suggested that an adequate local T-cell immune response is crucial for an effective antichlamydial T-cell immunity and also that microbial load could overwhelm the T-cell immunity against chlamydial infection.

Specificity of MoPn inhibition in polarized epithelial cells by T-cell clone 2.14-0. Since protective T-cell clone 2.14-0 is biovar specific, that is, is specific for MoPn but not GPIC or *C. trachomatis* serovar E, we investigated whether its chlamydial inhibitory action required MoPn stimulation; in addition, we sought to determine if the action was directed at MoPn exclusively or whether bystanding chlamydial agents could be inhibited by the activated clone. Polarized epithelial cells were infected with either MoPn or GPIC and cocultured with clone 2.14-0 that had been rested and then restimulated with UV-inactivated MoPn or GPIC EBs as antigens. An additional set of control cultures consisted of clone 2.14-0 plus APCs but no antigen. Figure 5 (left panel) shows that clone 2.14-0 could inhibit the growth of MoPn only when stimulated with the homologous antigen (MoPn) but not when stimulated with the heterologous antigen (GPIC). It is important to observe that unstimulated clone 2.14-0 (clone plus APC cultures) could not inhibit the growth of MoPn, indicating that specific antigenic stimulation is required for the MoPn-inhibitory action of the clone. Figure 5 (right panel) also shows that when clone 2.14-0 is appropriately activated with MoPn antigen, it is capable of inhibiting the productive growth of GPIC in polarized epithelial cells ($P < 0.0005$). There was no significant difference between the effect of clone 2.14-0 in the unstimulated state versus that when it was stimulated with GPIC and subsequently cocultured with MoPn- or GPIC-infected epithelial cells, indicating that clone 2.14-0 was not activated in either case. The results confirmed our previous observation that clone 2.14-0 is MoPn specific, requiring MoPn for activation, as measured by proliferative response and cytokine secretion (8). In addition, the requirement for specific antigenic stimulation for cytokine secretion by clone 2.14-0 also suggested that the mechanism of its antichlamydial action is probably via cytokine secretion which acts on both homologous and heterologous susceptible chlamydial agents in the vicinity of the T cell.

Role of epithelial cell–T-cell interaction in T-cell-mediated control of chlamydial growth in epithelial cells. In order to assess in more depth the nature of lymphoepithelial interactions required for T-cell control of chlamydial growth in epithelial cells, we analyzed the requirement for direct contact or close proximity of T cells and infected epithelial cells in the MoPn-inhibitory action of clone 2.14-0. Polarized epithelial cell cultures were infected with MoPn and then exposed to clone 2.14-0 either by coculturing or by separating the two cells with 0.45-, 3.0-, or 8.0-μm-pore-size filters. After 48 h of incubation, MoPn growth was assessed by tissue culture isolation. The results revealed that the separation of infected epithelial cells from the T-cell clone by a filter with 0.45-, 3.0-, or even 8.0-μm pores abrogated the MoPn-inhibitory action of the clone (data not shown). The results indicate that close proximity or possibly direct physical contact between infected epithelial cells and chlamydia-specific T cells may be required for T-cell action against chlamydial multiplication in epithelial cells.

DISCUSSION

A PELC system was employed to investigate the ability of chlamydia-specific T cells to control chlamydial growth in epithelial cells. The adaptation of the polarized epithelial cell culture system (46) to testing the antichlamydial function of specific T cells is an important step in applying an in vitro

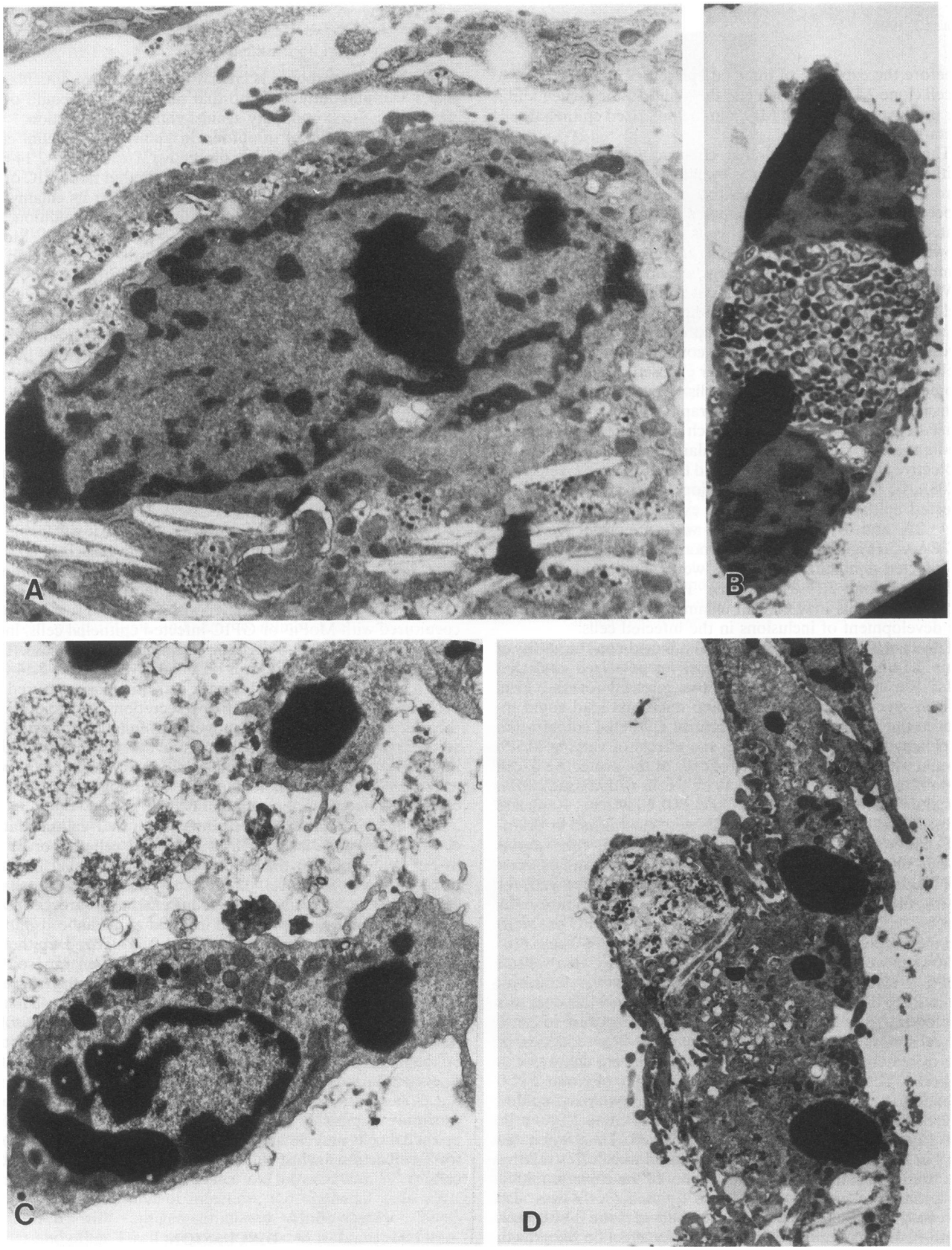

FIG. 2. TEM study of the morphology of chlamydiae and infected epithelial cells following exposure to protective and nonprotective T-cell clones. MoPn-infected polarized epithelial cells were exposed to protective and nonprotective T cells for 48 h. At the end of the incubation period, the filters containing the infected epithelial cells were washed and fixed in situ with 2% glutaraldehyde–0.5% paraformaldehyde in 0.1 M cacodylate buffer for 45 min at 37°C. Fixed filters were cut out and dipped in 2% molten Noble agar to enrobe them. Samples were then sectioned and processed for TEM as previously described (46). Polarized epithelial cells infected with MoPn in the absence of T cells (A and B) or the presence of protective clone 2.14-0 (C) or an irrelevant clone, TC08AA-5 (D), are shown. Magnifications: A, ×2,000; B, ×7,000; C, ×7,000; D, ×3,000.

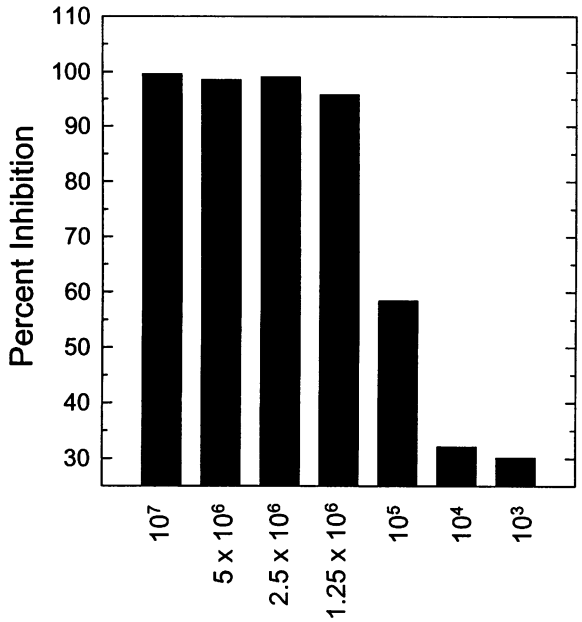

FIG. 3. Effect of T-cell numbers on the ability of clone 2.14-0 to inhibit the growth of MoPn in polarized epithelial cells. Duplicate cultures of polarized epithelial cells were infected with a constant inoculum of MoPn (10^3 IFU per well) and exposed to various numbers of clone 2.14-0 cells (10^7 to 10^3 per well) for 48 h. MoPn replication was determined by tissue culture isolation and detection of the inclusion bodies by the microimmunofluorescence procedure. Results are expressed as percent inhibition as defined in the legend to Fig. 1.

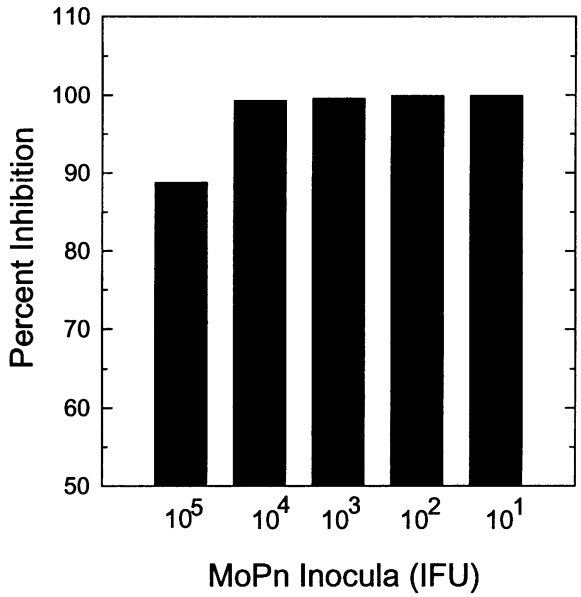

FIG. 4. Effect of MoPn inocula on the ability of clone 2.14-0 to inhibit the growth of MoPn in polarized epithelial cells. Duplicate cultures of polarized epithelial cells were infected with various inocula of MoPn (from 10^5 to 10^1 IFU per well) and exposed to a fixed number of clone 2.14-0 cells (2.5×10^6 per well) for 48 h. MoPn replication was determined by tissue culture isolation and detection of the inclusion bodies by the microimmunofluorescence procedure. The control culture was infected with 10^3 IFU of MoPn but no T cells. Results are expressed as percent inhibition as defined in the legend to Fig. 1.

system to reproduce an in vivo function within a close approximation.

Of the T-cell preparations tested in these studies, only in vivo-protective T-cell lines and clones could inhibit the productive growth of chlamydiae in polarized epithelial cells in vitro. Also, T cells alone in the absence of B cells were responsible for chlamydial control in this system, which corroborated previous results obtained from in vivo studies (25, 26, 31, 43–45), namely, that in the murine-MoPn model of chlamydial disease, T cells can mediate antichlamydial immunity. The present finding with polarized epithelial cultures may be analogous to the neutralization of chlamydial infectivity in vitro with specific polyclonal and monoclonal antibodies (15, 16, 47). However, the PELC system provides a more physiologically relevant approach to immune control of chlamydial control because T-cell function is tested in response to immunological stimulation by a specific antigen. It is important that only T cells capable of transferring protective immunity against MoPn genital infection in vivo can inhibit the growth of MoPn in the PELC system. Thus, if protective T cells employ identical antichlamydial mechanisms in vivo and in vitro, then the PELC system could provide a suitable model to investigate the mechanism of chlamydial control by T-cell-mediated immunity, which would have in vivo relevance. Indeed, unlike most frequently used in vitro systems that directly test the effects of various T-cell-derived components and products on microbial growth in culture, the present system would provide information on the cellular and molecular requirements for chlamydial control by the immune system.

Additional information derived from these studies is that not all T-cell clones may be capable of antichlamydial action and, since different clones may recognize distinct T-cell epitopes, it appears that not all T-cell epitopes would elicit protective T-cell immunity. The observations that not all antichlamydial T-cell clones could cure genital chlamydial infection when adoptively transferred into infected mice (8) and that not all defined T-cell epitopes of chlamydial (1, 39) or streptococcal (14, 41) antigens could provide help for antibody synthesis in vivo corroborate this point. The significance of distinguishing protective and nonprotective T-cell epitopes in peptide vaccine development cannot be overemphasized. Furthermore, if in vitro antichlamydial action of T cells is a reflection of in vivo ability to confer antichlamydial immunity, the PELC model system may provide a rapid in vitro method for testing protective T-cell clones as well as identifying protein antigenic fragments harboring protective T-cell epitopes by the ability of such fragments to stimulate chlamydia-immune T cells to inhibit chlamydial growth in epithelial cells. Such antigenic peptides containing protective T-cell epitopes may have peptide vaccine potential. The PELC system also provides a clean model to test the roles of the various immune cells, including natural killer cells, γ/δ T cells, purified B cells or T cells, and the subsets and subtypes, such as Th1 and Th2, in antichlamydial immunity.

Previous studies in this laboratory (29) and in others (20, 34, 42) have shown that specific cytokines, such as IFN-γ and TNF-α, elaborated during chlamydial infection, are involved in resolving the infection. Our present results appear to indicate that the mechanism of inhibition of chlamydial growth in epithelial cells probably involved close-range molecular and/or cellular interaction between T cells and infected epithelial cells. Close proximity between T cells and epithelial cells may be required for short-range action of antichlamydial cytokines elaborated by the T cells. The present findings further indicated that following immune elicitation in the serosal segment of the genital or ocular mucosa, the elicited T cells do not

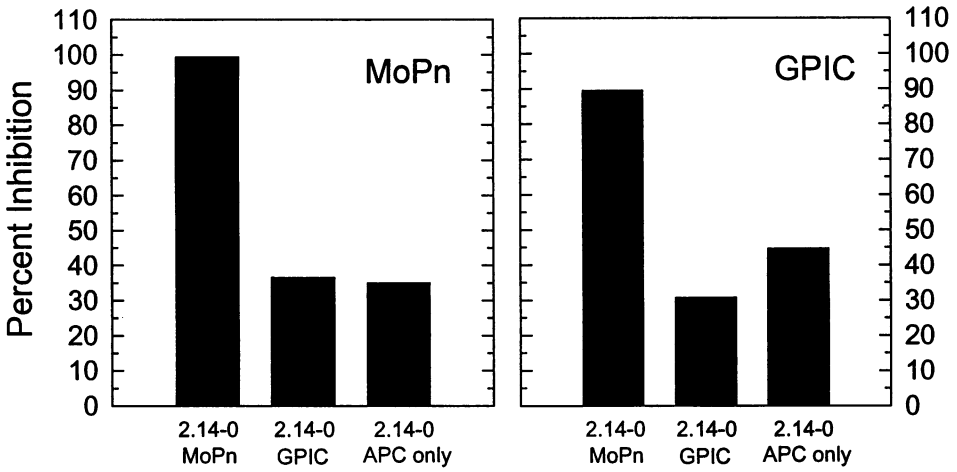

FIG. 5. Specificity of MoPn inhibition in polarized epithelial cells by T-cell clone 2.14-0. Polarized epithelial cells were infected with either MoPn or GPIC and cocultured with clone 2.14-0 that had been rested and restimulated with UV-inactivated MoPn or GPIC EBs. A set of cultures had nonstimulated clone 2.14-0. Both MoPn-infected (left panel) and GPIC-infected (right panel) cultures were incubated for 48 h, and the growth of the chlamydial agent was determined by tissue culture isolation. Control cultures had no T cells. Results are expressed as percent inhibition as defined in the legend to Fig. 1.

remain in the submucosal region but appear to migrate into the epithelium, interacting closely with infected epithelial cells, possibly via short-range-acting cytokines, before the infection can be terminated. The short-range activity of T-cell immune effectors against epithelial pathogens may underscore the need for local elicitation of mucosal immune responses to control mucosally acquired pathogens (2). A role for cytokines in mediating the antichlamydial action of T cells is suggested by results showing that specific antigenic stimulation is required for both cytokine secretion (8, 9) and antichlamydial action (Fig. 5).

Specific lymphoepithelial interactions associated with genital or ocular exposure to chlamydiae have been reported (22, 23, 38). These interactions were implicated in chlamydial control because of the absence of inclusions in areas of the epithelium with detectable association between epithelial cells and immigrant lymphocytes (22). It therefore appears that close interactions between epithelial cells and immune T cells are required for antichlamydial action of T cells elicited against chlamydial infection. Lymphocyte activity in mucosal epithelia has been shown to be antigen driven (5) and is predominantly T-cell activity (33), even during chlamydial infection (22). Apart from pathogen control, T cells are also involved in the regulation of epithelial cell differentiation and function, which may be mediated at least by IFN-γ (35). Other epithelial events influenced by T cells include the increase of major histocompatibility complex class II antigen expression and enhanced production and expression of the secretory component used for secretory immunoglobulin A transportation into exocrine fluids (35). Since most of these T-cell functions are cytokine mediated (3), lymphoepithelial interactions leading to modulation of epithelial activity or immune control of epithelial colonization by mucosa-routed pathogens may require close proximity of T cells and epithelial cells for the efficacy of short-range-acting cytokines.

In addition to the need for close proximity between T cells and infected epithelial cells, these studies also indicated that the continuous presence of an adequate T-cell response (possibly a high frequency of antigen-specific T cells) in the mucosal environment is required to ensure resolution of disease and obviously immunity to reinfection. Inadequate T-cell activity in the epithelium, as in the case of decreasing the number of T cells in culture, reduces T-cell control of chlamydial colonization and growth in the epithelium and may explain the inability of athymic *nu/nu* mice to resolve genital chlamydial infection (30). This observation is further supported by a previous report that susceptibility to chlamydial genital infection was associated with the disappearance of chlamydia-specific T cells in the genital mucosae (10). It appears that during the course of chlamydial infection specific ligand-receptor interactions between T cells and epithelial cells are required to ensure that the T cells remain in close proximity to the infected epithelial cells in order to ensure an effective T-cell function. The molecular mechanism of such interaction probably depends upon the presence of chlamydial antigens and specific signaling or homing molecules (18), among other features.

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