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Identification of *Chlamydia trachomatis* Antigens by Use of Murine T-Cell Lines

P. ROBERT BEATTY[1] AND RICHARD S. STEPHENS[2]*

Department of Biomedical and Environmental Health Sciences, University of California at Berkeley, Berkeley, California 94720,[1] and Department of Laboratory Medicine and the Francis I. Proctor Foundation, University of California at San Francisco, San Francisco, California 94143-0412[2]

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Chlamydia-specific short-term T-cell lines were used in conjunction with immunoblot techniques to examine *Chlamydia trachomatis* proteins for T-cell-stimulatory activity. This study was undertaken because of the known role of T cells in the resolution and pathogenesis of chlamydial infections. Therefore, determination of which chlamydial proteins are T-cell antigens and whether they evoke protective immunity or contribute to immunopathology is crucial. Immune lymph node cells were stimulated with whole chlamydial organism (elementary body) to derive predominantly CD4$^+$ T-cell lines. Proteins from the elementary body and the outer membrane and cloned proteins were examined for antigenicity with these T-cell lines in a proliferation assay. Although a majority of the elementary body protein fractions were positive in this assay, only four of the outer membrane fractions were stimulatory. The cloned major outer membrane protein and outer membrane protein 2 were stimulatory in the assay and may account for the reactivity in three of the four positive outer membrane fractions. The *C. trachomatis* heat shock protein 60, examined because of its putative role in causing delayed-type hypersensitivity, was found to stimulate the CD4$^+$ T cells. This approach with short-term T-cell lines with polyclonal reactivity was sensitive and specific in identifying chlamydial proteins as T-cell antigens.

Chlamydia trachomatis is a human pathogen that infects and causes disease in the ocular, genital, and respiratory mucosa. Recurrent or persistent infection of the conjunctiva with *C. trachomatis* can lead to the blinding eye disease trachoma. Serious sequelae from infections of the genital tract include epididymitis, pelvic inflammatory disease, ectopic pregnancy, and sterility. Although host immune responses to chlamydiae are poorly understood, chlamydia-specific T-cell responses are pivotal in mediating immunopathology as well as immunity to reinfection. Acute chlamydial infection elicits inflammatory responses initiated by nonspecific mechanisms and maintained by a specific cellular immune response. Grayston et al. (12) observed more-severe disease in vaccinated humans and in monkeys challenged with a heterologous *C. trachomatis* strain. Thus, pathology may result from a recurrent infection and is thought to be mediated by immune mechanisms. Morrison et al. (19) identified a Triton X-100-soluble factor that contributes to ocular inflammation, now known to be the chlamydial GroEL (18) or heat shock protein 60 (Hsp60). This Hsp60 may stimulate a T-cell-mediated delayed-type hypersensitivity during chlamydial infections (18).

The essential role of T cells in chlamydial immunity has been demonstrated in experiments with nude mice infected with the mouse pneumonitis strain of *C. trachomatis*. Intranasally infected *nu/nu* mice have higher mortality than control *nu/+* mice (32). Control *nu/+* mice are also able to resolve a genital infection that in *nu/nu* mice develops into a long-term infection (23). Moreover, adoptive transfer of chlamydia-specific T-cell lines derived from *nu/+* mice enables *nu/nu* mice to resolve a genital infection (21). T-cell depletion studies in normal mice gave similar results (26). Furthermore, experiments in B-cell-deficient mice have shown that, without antibody, mice are still able to resolve chlamydial infection (22, 30). The levels of T-cell-initiated cytokines, such as gamma interferon and tumor necrosis factor alpha increase during chlamydial infections (7, 31). Although the role of these cytokines in vivo is unknown, in vitro they have profound biological effects on chlamydiae (7, 24). It is apparent that T cells mediate a variety of immune responses, and it will be important to determine which T-cell antigens play a protective role and which contribute to host-mediated pathogenesis.

Assays of T-cell proliferation to *C. trachomatis* organisms have previously been done with human peripheral blood lymphocytes obtained from subjects with clinical symptoms and noninfected controls. Brunham et al. (6) observed a correlation between T-cell reactivity to chlamydial antigens and isolation of infectious organisms. The only specific chlamydial protein that has been tested for in vitro T-cell proliferation is the major outer membrane protein (MOMP) (3, 28). Research has focused on T-cell immune responses to MOMP primarily because of its dominant antibody response and the in vitro neutralizing capacity of antibodies to MOMP (27, 33). Although protective antibodies are made to the exposed chlamydial surface, which is predominantly MOMP, T-cell antigens comprise processed peptide sequences and may originate from the outer membrane or internal chlamydial proteins.

The unique disulfide cross-linked outer membrane of chlamydiae facilitates its extraction with sarcosyl detergent. The sarcosyl-insoluble outer membrane complex (OMC) contains abundant amounts of three cysteine-rich proteins (8). The three proteins are MOMP, with a molecular weight of 40,000; outer membrane protein 2 (OMP2), which appears as a doublet in serovar L2 with a molecular weight of 60,000; and OMP3, with a molecular weight of 12,500. These three abundant proteins have been cloned and sequenced, but there are other proteins in this outer membrane preparation that have not been characterized. Examination of proteins from the entire chlamydial organism will be essential in

* Corresponding author.

assessing which proteins may be prominently involved in the specific cell-mediated responses.

This investigation surveyed *C. trachomatis* proteins for antigenicity to murine T-cell lines. In these experiments, we measured the stimulatory response of separated protein fractions from the entire organism and the isolated sarcosyl-insoluble outer membrane as well as the response to cloned chlamydial fusion proteins. This identification of chlamydial T-cell antigens by immunoblotting techniques should direct further examination of specific protein antigens and focus emphasis on the molecular characterization of T-cell immune responses to *C. trachomatis*.

MATERIALS AND METHODS

Mice. Female BALB/cBy mice were obtained from Jackson Laboratory, Bar Harbor, Maine.

Bacterial strains. *C. trachomatis* L2/434/Bu was grown in the L929 mouse fibroblast cell line. Suspension cultures of 10^8 L929 cells were infected with 10^9 inclusion-forming units of chlamydial elementary bodies (EB). After 48 h at 37°C, the cells were sonicated, and the infectious EB were purified from the cell lysate over a discontinuous 30 to 44% Renografin (Squibb Diagnostics, New Brunswick, N.J.) gradient as described previously (15).

Preparation of OMC. The chlamydial outer membranes were extracted from 2×10^7 EB suspended in 3 ml of 2% *n*-lauroyl sarcosine (sarcosyl; Sigma Chemical Co.) in phosphate-buffered saline (8). The suspension was sonicated three times for 15 s each and incubated at 37°C for 30 min. The sarcosyl-insoluble OMC portion was obtained by centrifugation at 100,000 × *g* for 30 min. The entire sarcosyl extraction procedure was repeated twice on the resulting pellet. The final pellet of OMC was stored at −20°C.

Fusion proteins. A fragment of the *omp2* gene was amplified by polymerase chain reaction (PCR) to make an OMP2-glutathione-*s*-transferase (GST) fusion protein. Template DNA for PCR was prepared from purified serovar B organisms. Serovar B EB were lysed in 1% sodium dodecyl sulfate (SDS) plus 100 μg of proteinase K per ml. The DNA was isolated by three equal-volume extractions with phenol and two chloroform extractions. Oligonucleotides were synthesized from the B sequence of the *omp2* gene with 5′ *Bam*HI and 3′ *Eco*RI restriction sites for cloning (2). The sequence for amino acids Arg-127 to Val-268 was amplified by using oligonucleotides 5′-AAA-GGA-TCC-CGC-TTA-GGA-CAA-GGC-GAA-AAG-AGT-3′ and 5′-CAC-GAA-TTC-TAC-GCT-TGC-TGT-ATT-TTT-ATG-T-3′. The resulting PCR product was purified with a Centricon-30 concentrator and cleaved with restriction endonucleases *Bam*HI and *Eco*RI. The amplified *omp2* sequence was cloned into the vector pGEX-2T (25). As described previously (2), a 342-bp *Sau*3A fragment of the *omp3* gene from serovar L2 was cloned into pGEX to make a GST fusion protein containing the majority of the OMP3 protein. The L2 serovar chlamydial GroEL (Hsp60) was expressed in pGEX-2T as described previously (9). The *omp1* gene cloned into plasmid pET11d (Novagen) for overexpression of MOMP in *Escherichia coli* BL21(DE3) has been described previously (14).

T-cell lines. Female 8-week-old mice were intradermally injected with 50 μl of a 1:1 mixture of heat-inactivated (60°C for 1 h) EB (75 μg) and complete Freund's adjuvant. Ten to 14 days later, the draining lymph nodes were harvested, and the cells were washed two times in Hanks balanced salt solution (HBSS). The cells were cultured at 4×10^6/ml in RPMI medium containing 0.5% mouse serum, 0.01 M 2-mercaptoethanol, and 5×10^3 heat-inactivated EB per ml. After 4 to 6 days at 37°C, the cells were cultured at a concentration of 10^6/ml in RPMI containing 10% fetal bovine serum supplemented with 5×10^6 syngeneic irradiated spleen cells (3,000 rads of ^{137}Cs) per ml and 7.0% culture supernatant from rat spleen cells stimulated with concanavalin A. Following the 4 to 6 days of culture without antigen, viable T cells were purified by centrifugation over Ficoll (density, 1.077)-Hypaque and used in T-cell proliferation assays.

FACS analysis. Cells were tested after the resting culture period. The viable T cells were washed and resuspended in 0.1% bovine serum albumin in HBSS to inhibit nonspecific binding. The cells were incubated for 30 min on ice with biotinylated anti-CD4 antibody. Excess antibody was removed by washing four times with cold HBSS. Streptavidin-phycoerythrin and fluorescein isothiocyanate-conjugated anti-CD8 were added to the cells and then incubated on ice for 30 min. After being washed in HBSS, the cells were fixed in 1% formaldehyde in HBSS and then analyzed on a Becton Dickinson FACScan fluorescence-activated cell sorter (FACS).

Antigen preparation. Proteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE). EB lysate, OMC lysate, or GST fusion protein (150 μg) was placed in a 20-mm-wide well for electrophoresis in a 12% polyacrylamide gel (12 by 12 cm). One-dimensional immunoblotting was performed by a method similar to that of Abou-Zeid et al. (1). The proteins were transferred onto nitrocellulose (Schleicher & Schuell, Keene, N.H.) by using transfer buffer (17 mM Tris, 98 mM glycine, 21% methanol) at 18 V for 20 h. After transfer of proteins to nitrocellulose, a 2-mm edge was cut off from each well and stained in amido black. The proteins on the transfer were then cut into 4-mm horizontal strips numbered 1 (high molecular weight) to 28 (low molecular weight) and stored at −20°C. Control strips were taken from the same paper with no transferred protein. To prepare antigens for use in the T-cell assays, a dry piece (6 mm by 4 mm) of each strip was dissolved with 300 μl of dimethyl sulfoxide, and 600 μl of 50 mM sodium carbonate-bicarbonate buffer (pH 9.6) was added dropwise during vigorous mixing, precipitating a fine particulate. The resulting flocculate was washed two times with 1 ml of RPMI, resuspended in 1.5 ml of RPMI with 10% fetal bovine serum, and stored at −20°C.

T-cell proliferation assay. Lymph node cells were used at a concentration of 4×10^5 per well. For assays with cell lines, 2.5×10^4 to 5×10^4 T cells and 10^5 irradiated syngeneic spleen cells were plated per well in 96-well U-bottomed plates. EB were tested at various dilutions, and the optimal concentration for maximal stimulation was found to be 1.5 μg/ml. For assays examining separated EB and OMC protein fractions, 100 μl of prepared nitrocellulose antigen was added to each of three wells. On the third day of the assay, 0.5 μCi of tritiated thymidine (specific activity, 6.7 Ci/mmol) was added to each well and incubated overnight. The cells were harvested onto glass fiber filters with a cell harvester (Cambridge Technology Inc., Watertown, Mass.), and [^{3}H] thymidine incorporation was measured by scintillation counting.

RESULTS

Short-term T-cell lines. We initially examined T-cell proliferation to separated chlamydial EB fractions by using immune spleen cells, enriched populations of nylon wool-purified T cells, and immune lymph node cells. The reactiv-

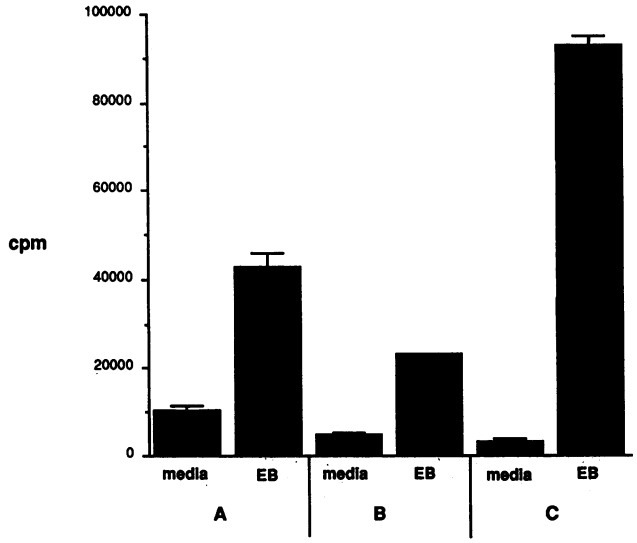

FIG. 1. T-cell proliferation assay. Comparison of T-cell proliferation to chlamydial EB with lymph node cells (LNC), a long-term T-cell line, and a short-term T-cell line. (A) LNC from immunized mice were plated at 4×10^5 cells per well. (B) A 12-week-old T-cell line, originally from LNC, stimulated biweekly with EB. (C) Two-week line from LNC with one stimulation cycle. (B and C) A total of 2.5×10^4 T cells and 5×10^4 irradiated syngeneic spleen cells were used per well. All cells were harvested on day 4 after an overnight incubation with 0.5 μCi of [^{3}H]thymidine. *C. trachomatis* L2 EB were used as test antigens at a concentration of 1.5 μg/ml. Medium alone was used in the control wells. The counts per minute (cpm) are reported as an average for triplicate wells ± SEM for each experimental value. Concanavalin A results for all three sets of cells were similar when evaluated on day 2 and were >100,000 cpm.

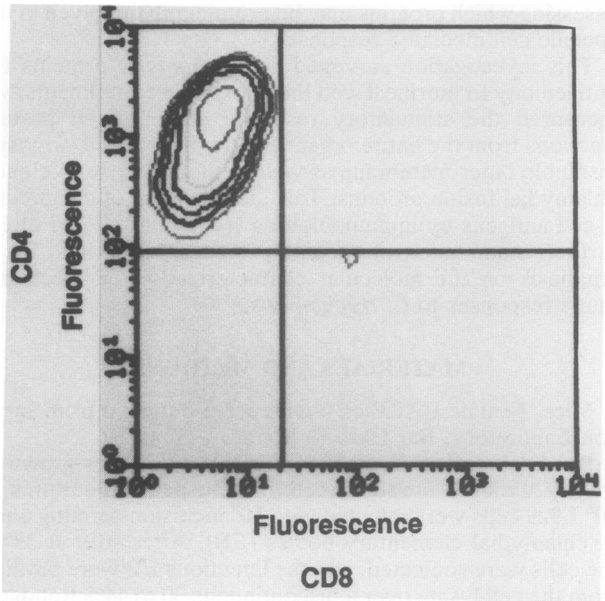

FIG. 2. FACS analysis of murine T-cell lines. Short-term chlamydia-specific T-cell lines were stained with biotinylated anti-CD4 and then with phycoerythrin-conjugated streptavidin and fluorescein isothiocyanate-conjugated anti-CD8. The cells were measured for fluorescence to each marker, with anti-CD4 represented on the ordinate and anti-CD8 represented on the abscissa.

ity of the different cell preparations to whole EB was evident, but the stimulation indices (SI) to antigen fractions were low because of high background proliferation in control wells without antigen. These T-cell assays with fresh immune spleen cells or lymph node cells from immunized or infected animals were not sufficiently sensitive to unequivocally measure specific protein antigenicity.

To increase the sensitivity of antigen detection, T-cell lines were derived from lymph node cells obtained from immunized animals. The lymph node cells were stimulated in vitro with chlamydial EB to enrich the cell population for chlamydia-specific T cells. As shown in Fig. 1, the short-term T-cell lines demonstrated a higher proliferative capacity against whole EB than a long-term (12-week) T-cell line and fresh immune lymph node cells. One stimulation with antigen followed by a culture period without antigen increased the specific reactivity to chlamydiae and decreased nonspecific proliferation.

FACS analysis of T-cell lines. Short-term T-cell lines were produced after stimulation of lymph node cells in vitro with inactivated chlamydial EB. This method should select T helper cells rather than cytotoxic cells because the antigens are presented via the exogenous class II pathway by antigen-presenting cells (APC) rather than the endogenous class I pathway. Helper T cells are usually identified by high expression of CD4. To characterize the cell population, we used FACS to evaluate short-term T-cell lines prior to use in T-cell proliferation assays. The results indicated that these short-term T-cell lines were reproducibly 97% $CD4^+$, with the remaining 3% being $CD8^+$ cells (Fig. 2).

T-cell immunoblotting. The EB proteins were electrophoretically separated into 28 protein fractions, transferred to nitrocellulose, prepared as antigens, and cultured with chlamydia-reactive T-cell lines to assess their in vitro stimulatory capacity. The relative chlamydial reactivity of each T-cell line was evaluated by the proliferation to whole chlamydial EB. The results of three different antigen preparations assayed with three different T-cell lines are shown in Fig. 3. The accuracy of this technique is demonstrated by the reproducible positive antigen fractions observed in assays with different protein preparations tested on identically prepared cell lines. The stimulatory EB protein fractions were found throughout the molecular weight spectrum. As many as 20 of the 28 chlamydial protein fractions demonstrated a stimulatory capacity in the assay, but only eight of the prepared nitrocellulose antigen fractions consistently had SI of greater than 3. Grouped areas of reactivity were found in the 40,000- to 70,000-molecular-weight range and the 28,000- to 34,000-molecular-weight range. Single positive fractions were found in the lower-molecular-weight spectrum located at fractions 22, 24, and 28. These short-term lines maintained broad specificity that made it easier to systematically examine proliferation to a wide range of chlamydial proteins.

Comparison of antigens in outer membranes and EB. After determining that there were numerous positive fractions in the EB protein profile, we decided to examine the proteins of the outer membrane for T-cell reactivity. The OMC of chlamydiae can be separated from the internal cell contents by using sarcosyl (8). This extracted OMC contains only a subset of the proteins found in whole EB preparations and is greatly enriched for the previously identified outer membrane proteins, including MOMP, OMP2, and OMP3. MOMP accounts for 60% of the protein in the outer mem-

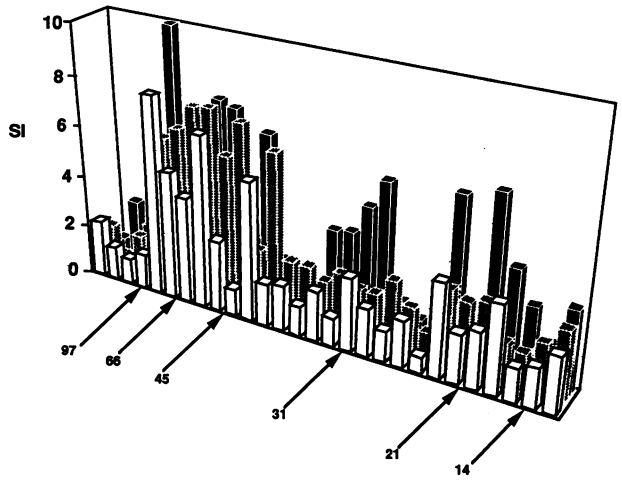

FIG. 3. T-cell proliferation to *C. trachomatis* proteins. T-cell proliferation was assessed on the 28 separated EB antigen fractions with short-term chlamydia-specific T cells and feeder cells as described in the legend to Fig. 1. Proteins were electrophoresed on SDS-PAGE gels and transferred to nitrocellulose sheets, and fractions were dissolved with dimethyl sulfoxide into a fine particulate for APC uptake. The results are shown as SI for each of the nitrocellulose strips in three different assays. The SI is equal to the average of triplicate experimental wells divided by the paper control value average of three wells. This three-dimensional plot of the data exhibits three different assays. Molecular weight markers (in thousands) are indicated.

brane (8) and, along with OMP2, can be readily distinguished on a Coomassie blue-stained gel.

OMC and EB lysates were electrophoretically separated on SDS-PAGE gels and prepared as antigens as described above. The antigen fractions were tested for comparison of stimulatory capacity in a T-cell proliferation assay with T-cell lines produced against EB. In Fig. 4, the SI are shown for the 28 antigen fractions of separated EB or OMC as they correspond to a Coomassie blue-stained protein profile. In Fig. 4B, there are four OMP fractions with significant SI, corresponding to molecular weights of 62,000, 58,000, 42,000, and 21,000. The fractions apparently containing the bands for MOMP and the OMP2 doublet were positive for in vitro proliferation.

A potential concern of this method is that protein quantity may be the determining factor in the measurement of relative SI, so that only abundant proteins score positive in this assay. Abou-Zeid et al. (1) demonstrated that as little as 0.05 μg of nitrocellulose particulate protein was stimulatory in a T-cell proliferation assay and equivalent to the amount of soluble antigen needed to elicit T-cell responses in vitro. In our assay, there were many positive fractions that were not seen as major protein bands by Coomassie blue staining (Fig. 4). Therefore, large amounts of protein did not necessarily correlate with T-cell reactivity, and proteins not detectable by staining may account for the T-cell proliferation.

MOMP and GST fusion proteins. We examined cloned chlamydial proteins for their ability to elicit T-cell proliferation in this assay. A MOMP immunoblot was obtained from a cell lysate by using an *E. coli* recombinant that overexpresses chlamydial MOMP (14). GST fusion proteins containing large portions of OMP2 and OMP3 and the entire Hsp60 were electrophoretically blotted onto nitrocellulose and tested in T-cell proliferation assays. As shown in Fig. 5, MOMP, OMP2, OMP3, and Hsp60 stimulated the chlamydia-specific T-cell lines. GST alone did not stimulate them and served as a negative control for the fusion proteins, as GST does not contain T-cell determinants recognized in BALB/c mice (10).

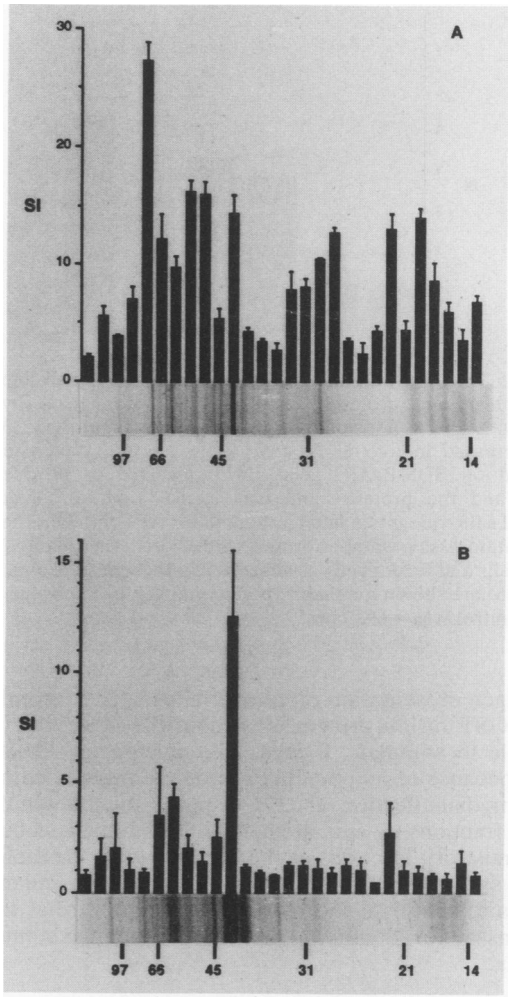

FIG. 4. T-cell proliferation assay comparing EB (A) and OMC (B) antigen fractions. The EB and OMC proteins were separated into 28 parallel antigen fractions for evaluation in T-cell proliferation assays. The SI for each of the 28 EB fractions (A) and the OMC fractions (B) are shown. Proteins were electrophoresed on SDS-PAGE gels and transferred to nitrocellulose sheets, which were cut and dissolved with dimethyl sulfoxide into a fine particulate for APC uptake. The T-cell proliferation assay was performed with short-term chlamydia-specific T cells and feeder cells as described in the legend to Fig. 1. The SI for each fraction is equal to the average value for three antigen-containing wells divided by the average value for control wells ± SEM. The Coomassie blue-stained protein profile for each preparation is shown below, along with molecular weight markers (in thousands) as indicated.

The responses to cloned proteins observed were essentially consistent with proliferative responses to the EB and OMC proteins. MOMP had the highest SI, which correlates with the outer membrane data. The T-cell stimulation by MOMP and OMP2 suggests that these proteins account for the proliferation to three of the OMP fractions. The OMP3-GST fusion protein was positive, and yet the OMC fraction containing OMP3 was not stimulatory. The native OMP3 is a lipoprotein (2, 11), and perhaps it is not as readily available

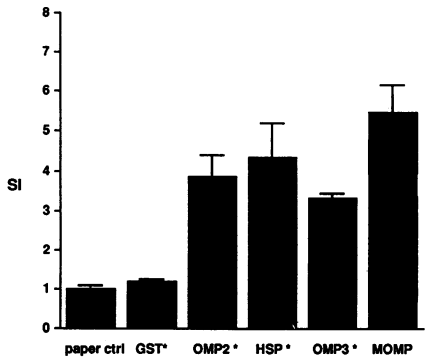

FIG. 5. T-cell proliferation assay with murine T-cell lines with cloned chlamydial proteins. Fusion proteins were made by using pGEX with GST as a fusion partner. *, GST fusion proteins. MOMP was expressed in toto in *E. coli*. Cloned proteins were electrophoresed on SDS-PAGE gels and transferred to nitrocellulose sheets, and the protein band was excised and dissolved with dimethyl sulfoxide into a fine particulate for APC uptake. The T-cell proliferation assay was performed with short-term chlamydia-specific T cells and feeder cells as described in the legend to Fig. 1. The SI ± SEM are shown for each experimental value. The value for the paper control was 4,692 cpm.

for antigen presentation on nitrocellulose. The recombinant OMP3-GST fusion protein is without the lipid moiety and was able to stimulate T cells. The chlamydial Hsp60 was tested because of its potential role in the immunopathology of chlamydial infection (18, 29). Hsp60 is abundant in the EB protein fractions (5) and, along with OMP2, may account for the stimulatory fractions at the higher end of the molecular weight spectrum. The ability of these known chlamydial proteins to stimulate short-term T-cell lines verifies the use of this assay for identifying previously unknown stimulating antigens.

DISCUSSION

The role of T cells in the immune response to *C. trachomatis* infections has only recently been examined. In animal models, T cells are essential mediators of protective immune responses to chlamydial infections. In nude mice, a genital infection remains unresolved, while in B-cell-deficient mice with intact T-cell function, chlamydial infection is controlled (22, 23). Depletion of $CD4^+$ cells leads to increased levels of chlamydiae and increased pathology (17). A role for $CD4^+$ T cells is also implicated by the increase in the levels of the T-cell-initiated cytokines gamma interferon and tumor necrosis factor alpha during infection (7, 31). Thus, T-cell-dependent immune mechanisms are essential for disease resolution, but their exact role is undefined, and the antigens eliciting protective or pathogenic immune responses are unknown.

Chlamydiae are intracellular bacteria that replicate only inside a host cell; however, a role for CD8 cell-mediated killing is unclear. Chlamydial proteins have not been detected on the surface of infected cells, but requisite processed peptides could still potentially be presented in the context of class I major histocompatibility complex. Mouse pneumonitis-specific, mostly $CD8^+$ T-cell lines were found to be protective in adoptive transfer experiments with nude mice (21). Lammert (16) found cytolysis in vitro of chlamydia-infected target cells, but only with culture times long enough for the chlamydiae to lyse the cells independent of $CD8^+$ cell-mediated cytotoxicity. Pavia and Schacter (20) showed no killing in a standard ^{51}Cr release assay. Thus, no cytotoxic T-cell response has been confirmed, and any role for $CD8^+$ cytotoxic or suppressor T cells remains enigmatic.

In this study, short-term murine T-cell lines were produced against the extracellular EB form of *C. trachomatis*. Stimulating the lymph node cells from immunized animals with antigen in vitro enriched the cell population for chlamydia-reactive T cells. Inactivated EB processed by autologous B cells and macrophages will be presented with class II major histocompatibility complex and therefore stimulate $CD4^+$ cells. FACS analysis results indicated that these 2-week-old T-cell lines were 97% $CD4^+$, with the remaining 3% being $CD8^+$ cells. By using a cell line after one stimulation-rest cycle, we maintained a polyclonal cell population with broad reactivity. These lymph node-derived T-cell lines demonstrated higher specific proliferative capacity when assayed against EB than did fresh lymph node cells or long-term T-cell lines. We recently produced short-term MOMP-specific T-cell lines which resulted in higher reactivity to the immunodominant epitope (13). This supports our hypothesis that this experimental design enriches for chlamydia-reactive T cells and selects for reactivity to immunodominant proteins.

These chlamydia-specific T-cell lines were used in proliferation assays to measure the reactivity to EB and OMC proteins as prepared antigens on nitrocellulose paper. We sought to determine whether *C. trachomatis* proteins provided good stimulation of specific T cells in vitro and which proteins were antigenic. The EB antigens demonstrated that many chlamydial proteins were stimulatory to T cells. The reactivity was concentrated in higher-molecular-weight areas, where more chlamydial proteins are located and certain previously identified protein antigens are found. The OMC fraction contained four antigen fractions exhibiting significant T-cell reactivity. The dominant responses were found in fractions containing MOMP and two processed forms of the OMP2 protein with molecular weights of 62,000 and 60,000 (4). T-cell specificity to MOMP, OMP2, and OMP3 was confirmed by transfers of each protein cloned in *E. coli*.

The only chlamydial protein previously identified as a T-cell antigen and shown to contain T helper cell epitopes is MOMP (3, 28). Our present results confirm that MOMP causes in vitro T-cell proliferation and implicate MOMP and OMP2 as major components of the T-cell-mediated immune response.

The T cells used in the assay were stimulated with antigens processed by macrophages and B cells in the lymph nodes and spleen. In vivo immunization followed by in vitro stimulation with whole EB was used to measure peptides that require processing before presentation. A mucosal infection or immunization at different sites may encounter other APC, such as dendritic cells, and might yield different results. Further experiments with T cells from chronically infected animals or humans may be strategies to test these possibilities.

We have shown that chlamydia-specific T-cell lines can be used to identify and characterize the T-cell response to chlamydial proteins. These short-term lines maintain broad specificity and should correlate more closely to the in vivo response than long-term lines or clones. The benefits of using cell lines are not only improved specificity but increased sensitivity in the assay for identifying T-cell antigens. The proliferation of these $CD4^+$ short-term lines to a variety of proteins suggests that $CD4^+$ T cells are important in both immunosurveillance and immunopathology. In adop-

tive transfer experiments, short-term lines driven by specific antigens (i.e., MOMP or Hsp60) could be used to evaluate T-cell-specific responses in differentiating between protection and pathogenesis. In addition to reaffirming the importance of outer membrane proteins, we have implicated other uncharacterized proteins as potent T-cell antigens. This evaluation of the stimulatory capacity of the protein spectrum of chlamydiae will help to direct further research on the T-cell response during infection.

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