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J Immunol 2000; 165:7285-7292; doi: 10.4049/jimmunol.165.12.7285
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Direct Detection and Magnetic Isolation of Chlamydia trachomatis Major Outer Membrane Protein-Specific CD8+ CTLs with HLA Class I Tetramers

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We recently identified HLA class I-presented epitopes in the major outer membrane protein (MOMP) of Chlamydia trachomatis that elicit CTL responses in human genital tract infections. T cells possessing cytolytic activities specific for these epitopes could be detected following in vitro stimulation of peripheral blood CD8+ T cells with peptides. In the present study we used HLA-A2 tetramers for detailed characterization of MOMP-specific CTL responses. Ex vivo tetramer analysis detected MOMP-specific T cells in the peripheral blood of infected individuals at significant frequencies (0.01–0.20% of CD8+ T cells). After in vitro stimulation with peptides, the frequencies of MOMP peptide-specific T cells increased up to 2.34% of CD8+ T cells in bulk cultures. In contrast, HLA-A2/MOMP tetramer-binding T cells were virtually undetectable in the peripheral blood from uninfected individuals, either ex vivo or after 3 wk of in vitro peptide stimulation of their T cells. Magnetically sorted, tetramer-bound T cells specifically lysed peptide-pulsed targets as well as C. trachomatis-infected epithelial cells with nearly 50-fold greater per cell efficiency than that of unsorted populations. This study provides conclusive evidence of in vivo induction of HLA class I-restricted CD8+ CTL responses to C. trachomatis MOMP. Direct detection of these cells with tetramers will allow their further characterization without prior manipulation and facilitate monitoring of CTL responses during infections and in immunization trials with MOMP-based vaccines. The Journal of Immunology, 2000, 165: 7285–7299.

Chlamydia trachomatis (Ct) is the most common cause of bacterial sexually transmitted disease (STD) worldwide (1) and of ocular trachoma in developing countries (2). A vaccine against Ct would have many health benefits and may become critical as antibiotic-resistant mutants appear (3). With regard to devising immunological interventions, the most puzzling and challenging aspect of chlamydial infections of humans is that despite evidence for diverse Ab (4–8) and T cell responses (9–18), reinfection is very common, leading to costly, widespread immunopathology. Studies showing that initial and subsequent infections are often caused by different serovars (i.e., serologically defined variants of Ct) have led to the current view that immunity to Ct is probably serovar specific (19, 20). This and the fact that neutralizing and serovar-defining Abs for Ct are uniquely directed to the major outer membrane protein (MOMP) (21, 22) have made MOMP an important focus of research aimed at understanding protective immune responses in Ct infections.

MOMP comprises five membrane-spanning constant domains (or segments) consisting of conserved amino acid sequences that alternate with four exterior variable domain loops containing sequences that are highly variable among serovars (21). All known B cell epitopes in MOMP have been mapped to variable domains, which have consequently emerged as a potential molecular basis for serovar-specific immunity. However, there is good evidence that T cells also play a critical role in host resistance to Ct. Studies with knockout mice and adoptive transfer have implicated both CD4+ and CD8+ T cell responses as important defense mechanisms (23–26). While the exact functions of these T cells that contribute to resolution of infections are not clearly understood, the effectiveness of T cell responses would partly depend on whether an infected epithelial cell can present T cell epitopes in association with MHC molecules. At present, the intravacuolar replication of Ct challenges our understanding of how its Ags would be processed by infected cells for presentation to T cells. Ct enters its host cells (mucosal epithelial cells of susceptible tissues) surrounded by a membrane-bound vacuole (27, 28), which neither fuses with the cell’s lysosomes nor acidifies. Ct replicates within the vacuole until the cell spontaneously lyases, liberating infectious progeny (29). It is not clear how Ct Ags could gain access to either the MHC class I (cytosol) or class II Ag processing (endolysosome) compartments during its infection of epithelial cells. Despite this unknown, we have identified about 30 HLA class II-restricted Th cell epitopes (13, 14) and eight HLA class I-restricted CTL epitopes in MOMP (12, 30) that are commonly recognized by subjects who have genital tract infections with Ct. These T cells, if present at sites of infection in vivo, could contribute to resolution and/or immunopathology of Ct infection. The great majority of Th and CTL epitopes we identified in MOMP are localized in constant

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0022-1767/00/$02.00
domains of MOMP (12, 13, 30) and could play an important role in providing cross-serovar protection. In light of apparent serovar-specific immunity against Ct, it is of particular interest that we identified several serovar-specific Th and CTL epitopes localized in the variable domains of MOMP (12–14). T cell responses directed to the variable domains of MOMP, in addition to neutralizing Abs, could contribute to serovar-specific immunity.

This paper is focused on the question of whether the MOMP-specific T cells we have previously described are actually elicited by human genital tract infections with Ct and could be monitored in vivo. Our previous study (12) concerned CTLs that were characterized only after peptide-stimulated proliferation of cells in vitro. Such experiments are subject to the caveat that the characterized cells are not representative of cells that are present in vivo in infected subjects or might even result from in vitro expansion of T cell populations that are present in uninfected individuals, i.e., might not represent responses to infection. In the present study we used fluorochrome-conjugated HLA-A2 tetramers (31) synthesized with MOMP CTL epitopes to answer this caveat. Flow cytometry allowed us to enumerate MOMP-specific CD8+ T cells in the peripheral blood of Ct-infected subjects directly ex vivo or after brief in vitro stimulation. Such cells were virtually undetectable in peripheral blood from uninfected subjects, suggesting that MOMP-specific CTLs detected ex vivo or after in vitro stimulation are characteristic consequences of genital tract infections with Ct.

We adapted a magnetic sorting procedure to conveniently isolate tetramer-bound T cells for further functional analysis. The magnetically sorted populations had potent and specific lytic activity against cells presenting MOMP CTL epitopes. Thus, the present study describes valuable tools for characterizing CTL responses in Ct infections, for monitoring such responses in vaccine trials, and for analyzing questions about the immunobiology of Ct infections. While HLA class I tetramers have been used to investigate anti-viral CTL responses (31–33), the present report concerns human infections with a vacuolar bacterial pathogen. Furthermore, unlike the commonly studied viruses, EBV and HIV-1, that persistently infect lymphoid cells, our tetramer analysis addresses CTL responses to an infectious agent that causes a localized mucosal infection.

Materials and Methods

Human subjects

STD subjects with a confirmed history of Ct-caused genital tract infection and uninfected subjects who lacked such a history had been identified and HLA-typed previously (12). Relevant clinical information about STD subjects is presented in Table I.

Synthetic peptides

Peptides corresponding to HLA-A2-restricted CTL epitopes, MOMP258 (258RILNMFTPY266) and MOMP260 (260MFTPYIGY268) (12), and HLA-B62-restricted epitope MOMP249 (249WQASLALSY257) (30) were synthesized at the University of Wisconsin Biotechnology Center (Madison, WI) as previously described (12). All three MOMP CTL epitopes are located in one of the four constant domains of MOMP. FluMP58 (58GILGFVFTL66), HLA-A2-restricted CTL epitope in influenza virus matrix protein M1 (34), was synthesized for use as a control.

Generation of peptide-specific CTLs and CTL assays

Bulk CTL cultures containing MOMP peptide-specific lytic activity were obtained from STD subjects as previously described (12). Briefly, CD8+ cells isolated from PBMCs using anti-CD8 magnetic beads (Miltenyi Biotech, Auburn, CA) were stimulated with peptide-pulsed, autologous adherent monocytes. Peptide-stimulated T cells were cultured in vitro in DMEM containing 10% pooled AB-negative human serum supplemented with recombinant human IL-7 (0.5 ng/ml) and IL-2 (25 U/ml). Two weeks after initiation, bulk cultures were restimulated with irradiated, autologous EBV-transformed lymphoblastoid cell lines (LCLs) that were preincubated with peptide. The resulting cultures are referred to as CTL-the name of the stimulating peptide.

Peptide-specific cytolytic activity of bulk cultures was assessed in 6-h [3H]uridine release assays (12). Cells used as targets include LCL 721 (HLA-A1, -A2, -B8, and -B51), LCL-45 (HLA-A2 and -B51), ME180 human cervical epithelial cells (HLA-A1, -A3, -B8, and -B44), and ME180(A2) (ME180 expressing HLA-A2 as a transgene) (12). Ct (serovar E)-infected ME180 cells were prepared as previously described (12) and were used in CTL assays at 40–48 h postinfection. The percent peptide-specific lysis was defined as (percent lysis of target in the presence of stimulating peptide − percent lysis of target in the absence of the peptide).

Table I. Frequencies of MOMP-specific CD8+ T cells in the peripheral blood of HLA-A2+ subjects who have had genital tract infections with Ct

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<th>Subject</th>
<th>HLA Type</th>
<th>Gender</th>
<th>Diagnosis*</th>
<th>Date PBMC Prepared (date of tetramer analysis)</th>
<th>% Tetramer+CD8+ T Cells</th>
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<td>B35</td>
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* Date of diagnosis of chlamydial genital tract infection and treatment with azithromycin.

ND: Not determined.

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<td>A2/MOMP260</td>
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<tr>
<td>HLA-A2</td>
<td>A2/MOMP258</td>
<td>A2/MOMP260</td>
</tr>
</tbody>
</table>

* Date of diagnosis of chlamydial genital tract infection and treatment with azithromycin.

ND: Not determined.
Preparation of HLA-A2/MOMP tetramers

HLA-A2 tetramers were prepared with CTL epitopes MOMP258 and MOMP260 using the previously described method (31). Briefly, β₂-microglobulin and a truncated form of HLA-A2 heavy chain (in which the transmembrane and cytosolic domains had been removed and a specific biotinylation site added to the C-terminus) (35) were expressed in *Escherichia coli* strains BL21 (DE3) lyS3; and inclusion bodies were purified as previously described (36). The purified HLA-A2 and human β₂-microglobulin were refolded at 10°C in the presence of 25 μg/ml of peptide (Research Genetics, Huntsville, AL) and protease inhibitors (pepsin A (1 μg/ml), leupeptin (1 μg/ml), and PMSF (0.4 mM)). Soluble monomeric complexes were purified by gel filtration over a Superdex 200HR column (Amersham Pharmacia Biotech, Piscataway, NJ) and enzymatically biotinylated by overnight incubation with purified BirA at room temperature with the following components: 5 μM HLA-A2/peptide, BirA enzyme (1.5 × 10⁵ U; Avidity, Denver, CO), 80 μM biotin, 10 mM ATP, 10 mM magnesium acetate, and 20 mM bicine. Unbound biotin was removed by gel filtration, and the purified monomers were tetramerized by incubation with PE-labeled streptavidin (Molecular Probes, Eugene, OR) at a molar ratio of 4:1. Finally, tetramers were purified by gel filtration taking fractions that represented a single peak and were kept in the dark at 4°C in PBS (pH 8.0) containing 0.02% sodium azide, 1 μM pepstatin, 1 μg/ml leupeptin, and 0.5 mM EDTA.

Tetramer analysis of T cells

Unstimulated PBMCs or peptide-stimulated CD8⁺ cells were incubated at 4°C for 45 min in staining buffer (2% FCS in Ca²⁺, Mg²⁺-free PBS) containing HLA class I tetramer-PE (typically at 1/100 dilution), anti-CD3-PE-Cy7, and anti-CD8-PE-FITC (Caltag, Burlingame, CA). Unfixed cells were washed twice with staining buffer and kept on ice until three-color flow cytometric analysis was performed using a FACSComp flow cytometer (Becton Dickinson, San Jose, CA). For PBMCs, 2–4 × 10⁶ cells were stained in a 100-μl volume, and 10⁶ total events were acquired. Frozen PBMCs were thawed and incubated at 37°C in DMEM containing 10% human serum for at least 24 h before analysis. For peptide-stimulated CD8⁺ T cells, 2 × 10⁶ to 1 × 10⁷ cells were stained in a 50-μl volume, and 1–5 × 10⁶ total events were acquired. Data analysis was performed using CellQuest software (Becton Dickinson). Lymphocytes were identified by forward and side angle light scatter. After gating on CD3⁺ CD8⁺ T cells, tetramer-bound T cells were enumerated by their PE-fluorescence. For ex vivo quantitation of HLA-A2/MOMP tetramer-binding T cells in STD subjects, at least two HLA-A2-STD subjects were included as negative controls in each tetramer analysis to establish nonspecific background staining. The staining of HLA-A2-STD subjects was typically 0.1–0.3% of CD8⁺ T cells, which was subtracted from the staining of HLA-A2-STD subjects to yield values for specific staining of HLA-A2-restricted MOMP258- and MOMP260-specific CTLs.

Magnetic sorting of HLA class I tetramer-bound T cells

Bulk CTL cultures were incubated with PE-tagged HLA-A2/MOMP tetramers at 4°C for 45 min and washed twice with PBS. Cells were incubated with anti-PE magnetic beads according to the manufacturer’s instruction (Miltenyi Biotec) by adding 20 μl of beads to <10⁶ tetramer-bound cells resuspended in 80 μl of PBS. After 15 min incubation at 4°C, cells were washed twice and passed through a magnetic column. Anti-PE bead-bound cells were positively selected and used as effector cells in CTL assays.

**Results**

**Specificity of HLA-A2/MOMP tetramers**

First we ascertained that the HLA-A2 tetramers prepared with CTL epitopes MOMP258 and MOMP260 (12) could specifically identify MOMP CTLs in vitro peptide-stimulated heterogeneous T cell populations. Fig. 1 shows the tetramer analysis of bulk CTL cultures obtained from an STD subject that had specific lytic activity for the HLA-A2-restricted epitope MOMP258 (i.e., CTL-MOMP258) and for the HLA-B62-restricted epitope MOMP249 (i.e., CTL-MOMP249) (30). The proportion of HLA-A2/MOMP258 tetramer-binding CD8⁺ T cells in the bulk CTL-MOMP258 culture increased from 0.07% on day 10 (Fig. 1A) to 0.43% on day 21 (Fig. 1B), correlating with a smaller relative increase in MOMP258-specific lytic activity, from 15 to 25%. However, the percent specific lysis does not always increase in direct proportion to the E:T cell ratio in bulk CTL populations, and not all cells that proliferated after in vitro stimulation may have retained cytolytic activity. Fig. 1, A and B, represent specific binding of HLA-A2/MOMP258 tetramers to T cells; tetramer-binding T cells were not detected in the same CTL-MOMP258 culture with an irrelevant tetramer, HLA-A2/MOMP260, made with an epitope that was not used for in vitro stimulation (Fig. 1, D and E). In addition, CTL-MOMP249, a T cell culture containing specific lytic activity for HLA-B62-restricted epitope MOMP249, lacked a T cell population that binds HLA-A2/MOMP258 tetramers (Fig. 1C).

Similarly, HLA-A2/MOMP260 tetramers could specifically identify an expanding CTL population in STD subject-derived bulk cultures stimulated with peptide MOMP260 (Fig. 2). The proportion of HLA-A2/MOMP260 tetramer-binding CD8⁺ T cells in the CTL-MOMP260 culture increased from 0.04% on day 10 to 0.36% on day 21 (Fig. 2, C and D), which correlated relatively well with the 5-fold increase in peptide-specific lytic activity from 3 to 15%. The tetramer-binding population was not detected in the same CTL-MOMP260 culture with an irrelevant tetramer HLA-A2/MOMP258 (Fig. 2, A and B). The specificity of HLA-A2/MOMP258 and HLA-A2/MOMP260 tetramers was further demonstrated by their lack of binding to CD8⁺ T cells that were stimulated in vitro with FluMP58 peptide (data not shown).
In all CTL assays we have performed to date, CTL cultures elicited with peptide MOMP258 did not recognize targets presenting peptide MOMP260 and vice versa (12). Therefore, peptides MOMP258 and MOMP260, overlapping by seven amino acids, were considered to be distinct CTL epitopes. This is now validated by the distinct staining of CTL-MOMP258 and CTL-MOMP260 cultures with tetramers HLA-A2/MOMP258 and HLA-A2/MOMP260, respectively, and by the lack of cross-staining between the two tetramers.

**HLA-A2/MOMP tetramer-binding cells are found in C. trachomatis-infected subjects, but not in uninfected subjects**

CD8+ T cells possessing MOMP258- and MOMP260-specific lytic activity were detectable in all 14 STD subjects, but in only one of seven HLA-A2-uninfected subjects we have tested to date (12, 30), implying that MOMP-specific CTLs found in STD subjects are most likely memory T cells that resulted from Ct genital tract infections. This inference is now reinforced by tetramer analysis.

Peripheral blood CD8+ T cells from subject STD205 and from three uninfected subjects who had previously tested negative for the presence of MOMP peptide-specific CTLs were stimulated in vitro with peptide MOMP258 or MOMP260 following the standard protocol. To clearly demonstrate the difference between infected and uninfected subjects, peptide-stimulated CD8+ T cells were allowed to expand up to 20 days before tetramer analysis. Representative data obtained with STD205 and one of the three uninfected recruits are shown in Fig. 3. While 0.92% of the CD8+ T cells in STD205-derived CTL-MOMP258 culture bound HLA-A2/MOMP258 tetramers (Fig. 3A), insignificant numbers of such cells were detected in the corresponding culture derived from the uninfected subject (Fig. 3B). Likewise, HLA-A2/MOMP260-binding T cells comprised 2.34% of the CD8+ T cells in STD205-derived CTL-MOMP260 culture (Fig. 3G), but were virtually undetectable in the corresponding culture of the uninfected subject (Fig. 3H). Again, the staining shown in Fig. 3, A and G, is specific, as demonstrated by the lack of detectable tetramer-binding cells when the same cultures were stained with irrelevant tetramers (Fig. 3, C and E). These data confirm that CTL precursors or CTLs specific for MOMP peptides present in the peripheral blood of STD subjects have indeed resulted from Ct infections. They also imply that the frequencies of naive, precursor T cells specific for MOMP must be quite low in uninfected individuals, lower than can be detected by tetramer analysis, even after almost 3 wk of in vitro peptide stimulation.

**Ex vivo quantitation of HLA-A2-restricted MOMP-specific CTL precursors**

Direct ex vivo staining performed with a group of 10 HLA-A2+ STD subjects revealed that 0.01–0.20% of peripheral blood CD8+ T cells are specific for a single CTL epitope, MOMP258, although such T cells were not clearly detected in two of the 10 STD subjects tested (Table I). The corresponding frequencies for epitope MOMP260 were 0.01–0.11%, with one of the nine tested subjects apparently lacking such T cells (Table I). Ex vivo frequencies of MOMP-specific CD8+ T cells did not necessarily correlate with
the interval between the diagnosis of infection and the sampling of blood for tetramer analysis, implying that the MOMP-specific CTLs represent a memory T cell pool that is maintained at a certain level after primary infection. As expected from the lack of tetramer-binding CTLs in in vitro stimulated T cell cultures (Fig. 3), HLA-A2/MOMP tetramer-binding T cells were essentially undetectable upon ex vivo staining of two uninfected recruits (data not shown).

Magnetic sorting of HLA-A2/MOMP tetramer-bound T cells of potent, specific lytic activity

While tetramer-bound T cells can be sorted by FACS, we reasoned that magnetic beads coated with anti-PE Abs could be used to enrich for T cells that bound PE-tagged tetramers. In an experiment shown in Fig. 4A, a 29.25% tetramer+ population was obtained by positive sorting with anti-PE beads from a starting population that was only 0.26% tetramer+. The negatively sorted population was 0.08% tetramer− (data not shown).

Magnetic sorting enriched for MOMP-specific CTLs and selectively eliminated CTLs of irrelevant specificity. Since our in vitro stimulation protocol involves restimulation with peptide-pulsed LCLs, bulk CTL cultures often show rather high background lysis of LCL targets in the absence of peptide (Fig. 4B; ~0.5% tetramer− CD8+ T cell population). This is probably due to the expansion during in vitro cultivation of high frequency EBV-specific CTLs present in most individuals (33, 37). Interestingly, this particular MOMP peptide-stimulated culture contained influenza virus FluMP58-specific lytic activity, although the T cells had not encountered FluMP58 peptide in vitro (Fig. 4B). This, too, may be due to in vitro bystander expansion of in vivo activated FluMP58-specific CTLs. After magnetic sorting with anti-PE beads, tetramer-bound T cells showed a significantly reduced background lysis of LCL targets and the absence of FluMP58-specific lytic activity (Fig. 4C). The per cell MOMP258-specific lytic activity of the sorted population was approximately 50-fold greater than that of unsorted cells; 25% specific lysis by sorted cells at an E:T cell ratio of 1 (Fig. 4C) compared with 27% specific lysis by unsorted cells at an E:T cell ratio of 50 (Fig. 4B). This increase in MOMP-specific lytic activity results from enrichment of MOMP-specific CTLs and not from enhanced lytic activity caused simply by binding of tetramers and anti-PE magnetic beads. When CTLs incubated with tetramers followed by anti-PE beads were used unsorted as effectors in CTL assays, they did not exhibit increased peptide-specific lytic activity compared with the same cells that were not exposed to tetramers and magnetic beads (Table III).

Sorted HLA-A2/MOMP tetramer-bound T cells specifically lyzed Ct-infected epithelial cells, confirming that the lytic activities of MOMP peptide-stimulated bulk CTL cultures against Ct-infected cells that we reported previously (12) indeed are of MOMP-specific CTLs. A bulk CTL-MOMP258 culture and HLA-A2/MOMP258 tetramer-bound T cells isolated from that culture were compared in CTL assays (Fig. 5). Both unsorted (~0.5% tetramer− CD8+ T cell population) and sorted cells lysed not only peptide-pulsed targets but also Ct-infected ME180 human cervical epithelial cells, with sorted T cells showing approximately 50-fold greater per cell killing efficiency than the unsorted T cell population. Similar results were obtained with CTL-MOMP260. Both unsorted (Fig. 6A; 2.34% tetramer− CD8+ T cell population; same cells as shown in Fig. 3G) and sorted populations (Fig. 6B) could lyse peptide-pulsed targets as well as infected targets, but only when the targets expressed HLA-A2, confirming the HLA allele specificity of CTL recognition. While sorted cells contained much greater per cell lytic activity than did unsorted cells, the population depleted of tetramer-binding cells barely had MOMP-specific lytic activity (Fig. 6C).

Because about 30 ml of blood was obtained per draw from a human subject, most bulk cultures we set up yielded <5 × 104

![FIGURE 4](http://www.jimmunol.org/)

Selective enrichment of MOMP-specific CTLs by magnetic sorting. A, A bulk CTL-MOMP258 culture derived from STD266 was incubated with HLA-A2/MOMP260 tetramers followed by anti-PE magnetic beads and analyzed by flow cytometry. The proportion of tetramer-bound lymphocytes in each sample is shown inside the dot plots. CTL assays were performed with an STD205-derived CTL-MOMP258 bulk culture (~0.5% tetramer− CD8+ population; B) and HLA-A2/MOMP258 tetramer-bound cells magnetically sorted from that culture (C) on day 9 of in vitro cultivation. Targets were [3H]uridine-labeled, HLA-A2+ LCL 721 cells preincubated with the indicated peptides.

![FIGURE 5](http://www.jimmunol.org/)

Lysis of *C. trachomatis*-infected cervical epithelial cells by enriched HLA-A2/MOMP258-bound T cells. After 12 days of in vitro peptide stimulation, an STD205-derived CTL-MOMP258 bulk culture (0.28% tetramer− CD8+ population) and HLA-A2/MOMP258-bound cells magnetically sorted from that culture were used as effector cells in CTL assays. Targets were the [3H]uridine-labeled human cervical epithelial cell ME180[A2] (i.e., ME180 expressing HLA-A2 as a transgene) preincubated with or without peptide MOMP258 or infected with Ct.
tetramer-bound T cells after anti-PE bead sorting. This made it difficult to perform both CTL assays and flow cytometric analysis with sorted T cells, so that postsorting flow cytometric analysis was often omitted and was not performed for the T cells described in Figs. 5 and 6. However, the data shown in Fig. 4A and the lack of MOMP-specific lytic activity in negatively sorted cells in Fig. 6C suggest that isolation of tetramer-bound T cells was quite efficient. Magnetic sorting could be a convenient, faster, and less expensive alternative to FACS, especially when working with CTL cultures containing relatively high frequencies of tetramer-binding T cells.

In summary, the fact that Ct-infected genital tract epithelial cells are susceptible to lysis by the enriched HLA-A2/MOMP tetramer-bound T cells solidifies a few points suggested by our previous studies (12): 1) CD8⁺ CTLs specific for MOMP peptides are indeed induced in genital tract infections with Ct; 2) although neither the organism nor MOMP has been found in the cytosol of infected cells, MOMP is clearly processed and presented by the host’s HLA class I molecules; and 3) if recruited to infected mucosal sites, MOMP-specific CTLs might have protective and/or pathological effects by destroying infected cells.

Discussion

HLA class I tetramers have been established as the most rapid and accurate means currently available for quantitative evaluation of even rare Ag-specific T cells. Our present study exemplifies the importance of defining minimal CTL epitopes and their HLA class I restriction elements for producing HLA class I tetramers to use in studies of CTL responses against Ct.

An important accomplishment of the present study is that it provides a firm basis for using HLA class I tetramers to identify and quantify MOMP-specific CTLs in many contexts. For instance, the tetramers could be used to monitor the outcome of immunizations with MOMP-based vaccines or to relate CTL frequencies to immunopathologic consequences of infection. These applications will be practical because all eight MOMP-specific CTL epitopes we have identified are presented by HLA class I allootypes commonly expressed in the general population (12, 30). Since seven of the eight MOMP CTL epitopes identified in genital tract infections contain sequences conserved in all Ct serovars, tetramers made with these epitopes could be used to assess whether the same epitope specificities are observed in ocular trachoma patients. It might be possible to design a MOMP epitope-based subunit vaccine that elicits protective CTL responses in the conjunctiva and the genital tract.

As determined by ex vivo tetramer analysis, the frequencies of peripheral blood CD8⁺ T cells specific for MOMP CTL epitopes are generally much lower than those reported in some viral infections (Table II). Even after 2–3 wk of in vitro peptide stimulation, usually <1% of all CD8⁺ T cells in bulk cultures derived from STD subjects bound HLA-A2/MOMP tetramers (Figs. 1–4 and data not shown), while as much as 5% of CD8⁺ cells in EBV-seropositive subjects bound HLA-B8/BZLF1 tetramers directly ex vivo (33). The low frequency of MOMP-specific CTL precursors could result from biological differences between viral and Ct infections. For instance, unlike our STD subjects who, to the best of our knowledge, did not have reinfection or persistent infection with Ct, most individuals probably have had multiple infections with influenza virus that could result in high frequencies of virus-specific CTLs (32). In the cases of EBV (33) and HIV-1 (31), persistent infection and sporadic reactivation of latent infection may provide repeated stimuli that trigger the expansion of Ag-specific T cells, accounting for their high ex vivo frequencies. In addition, the viruses shown in Table II cause different courses of infection than Ct. EBV and HIV-1 infection of B cells and T cells, respectively, become systemic and potentially capable of stimulating CTLs in various lymph nodes throughout the periphery.

Table II. Comparison of the frequencies of CD8⁺ T cells recognizing a single epitope in various human infections as determined by tetramer analysis

<table>
<thead>
<tr>
<th>Infection</th>
<th>Tetramer Used</th>
<th>Peptide-Specific CTLs (% of CD8⁺ T cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ct⁺</td>
<td>HLA-A2/MOMP258–266</td>
<td>0.01–0.20</td>
</tr>
<tr>
<td>Flu</td>
<td>HLA-A2/MOMP260–268</td>
<td>0.01–0.11</td>
</tr>
<tr>
<td>EBV</td>
<td>HLA-A2/BMLF1 280–288</td>
<td>0.4–1.1</td>
</tr>
<tr>
<td>HIV-1</td>
<td>HLA-A2/Gag 77–85</td>
<td>0.17–0.28</td>
</tr>
<tr>
<td></td>
<td>HLA-A2/Pol 309–317</td>
<td>0.02–0.77</td>
</tr>
</tbody>
</table>

*a Frequencies of HLA-A2-restricted CTLs specific for MOMP258–266 or MOMP260–268 in individuals with a previous history of chlamydial genital tract infection (present study).

*b Frequencies of HLA-A2-restricted CTLs specific for influenza virus FlmMP8–86 in healthy individuals (32), assuming 10% of PBMCs are CD8⁺ T cells.

*c Frequencies of EBV-specific CTLs in seropositive healthy adults (33).

*d Frequencies of HIV-1-specific CTLs in seropositive individuals not suffering from AIDS (31).
However, Ct infection of epithelial cells is mostly localized to mucosa, which could result in a more differential compartmentalization of memory T cells between different lymphoid tissues than would be expected in the case of systemic spread of infection (38, 39). For instance, it remains possible that more MOMP-specific CTLs could be found in the genital tract or genital tract-associated mucosal tissues than in the peripheral blood of STD subjects.

The relatively low frequency of MOMP-specific CTL precursors compared with that of anti-viral CTLs could also be due to our choices of epitopes to study. Because MOMP is the only Ct Ag for which extensive investigation of human CTL responses has been reported, it is not known whether MOMP CTL epitopes are immunodominant epitopes in genital tract infections. However, we should note that infected subjects regularly recognize MOMP CTL epitopes as long as they express appropriate HLA class I allotypes. It remains to be seen whether frequencies of CD8$^+$ CTL precursors greater than those for MOMP are elicited by other Ct Ags. Promising candidates include the Inc proteins that are localized on the Ct vacuolar membrane (40) and Cap1, which elicits murine CTLs that are protective after adoptive transfer (41).

One of the important questions in studies of immunity to mucosal pathogens is whether Ag-specific T cells found in peripheral blood are reliable indicators of the presence of such T cells at infected mucosal sites. The maintenance of a high frequency memory T cell pool at relevant tissue sites is one of the important features of protective immunity (42). Studies of HIV-1 (43) and HSV-2 (38, 44) infections in mice have indicated that long-lasting protection against viral challenge in the genital tract requires pools of memory CTLs that are elicited by mucosal inoculation and become localized in genital tract mucosa. Although it would be difficult to obtain suitable T cell samples, HLA class I tetramer-positive T cells are known to express a high level of 7 integrin and preferentially home to the gut (48–52). Whether homing pathways defined for gut-associated lymphoid tissues would be relevant to other mucosal sites is not clear. Although there is a report that $\alpha_4\beta_7$ is expressed by cytolytic CD8$^+$ T cells present in the vaginal epithelium of SIV-infected monkeys (53), expression of $\alpha_4\beta_7$, rather than $\alpha_4\beta_7$, is characteristic of murine T cells homing to Ct-infected genital mucosa (54). Evaluation of HLA-A2/MOMP tetramer-binding peripheral blood T cells may also yield clues to the tissue specificity and potential in vivo function of these T cells. Unlike memory T cells elicited by parenteral immunization, gut-derived memory T cells are known to express a high level of $\alpha_4\beta_7$ integrin and preferentially home to the gut (48–52). Whether homing pathways defined for gut-associated lymphoid tissues would be relevant to other mucosal sites is not clear. Although there is a report that $\alpha_4\beta_7$ is expressed by cytolytic CD8$^+$ T cells present in the vaginal epithelium of SIV-infected monkeys (53), expression of $\alpha_4\beta_7$, rather than $\alpha_4\beta_7$, is characteristic of murine T cells homing to Ct-infected genital mucosa (54). Evaluation of HLA-A2/MOMP tetramer-binding peripheral blood T cells for the expression of integrins such as $\alpha_4\beta_7$ or $\alpha_4\beta_7$ could provide useful information regarding the involvement of these molecules in homing of MOMP-specific lymphocytes to different mucosal tissues. Such determinations can be made without prior in vitro manipulations of T cells that might alter the phenotypes of the cells.

**Acknowledgments**

We thank Dr. Eric Pamer (Yale University School of Medicine, New Haven, CT) for his kind gift of the plasmid constructs expressing human $\beta_2$-microglobulin and HLA-A2 with a biotinylation signal. We also thank Dr. Natalie Marshall and Ingrid Pilip for technical advice.

**References**


**Table III. Binding of HLA-A2/MOMP tetramers followed by anti-PE beads does not stimulate otherwise noncytolytic CD8$^+$ T cells**

<table>
<thead>
<tr>
<th>Targets$^a$</th>
<th>None (E:T = 50)</th>
<th>HLA-A2/MOMP258 tetramers followed by anti-PE beads (E:T = 50)</th>
<th>HLA-A2/MOMP258 tetramers followed by anti-PE beads (E:T = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME180[A2]</td>
<td>1.4</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>ME180[A2]+MOMP258</td>
<td>14.6</td>
<td>13.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

$^a$ [3H]Uridine-labeled ME180[A2] cells preincubated with or without MOMP258 peptide were used as targets. Percent lysis of targets by indicated CTLs are shown.

$^b$ A bulk CTL-MOMP258 culture was derived from STD205 and had 0.35% HLA-A2/MOMP258 tetramer$^+$ CD8$^+$ T cells on the day of CTL assays (day 13 of in vitro peptide stimulation). Unsorted T cells that were incubated with or without HLA-A2/MOMP258 tetramers and anti-PE beads (see Materials and Methods) were used as effectors at indicated E:T ratios.


