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Primary human T-cell responses to the major outer membrane protein of *Chlamydia trachomatis*

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SUMMARY

The major outer membrane protein (MOMP) of *Chlamydia trachomatis* is the main candidate antigen for a synthetic vaccine against chlamydial infection. Antibodies to surface-exposed epitopes on MOMP neutralize chlamydial infectivity but little is known about T-cell recognition of the molecule. We have measured primary human T-cell responses to recombinant fragments of MOMP as well as to the whole organism and synthetic MOMP peptides. Using antigen-pulsed low density cells (LDC) we were able to stimulate proliferative responses with T cells from most naive individuals. This response was antigen dose dependent and displayed an absolute requirement for dendritic cells in the antigen-presenting cell (APC) population. Several T-cell epitopes were identified in MOMP and one which stimulated T cells from 80% of donors was resolved as a 12 amino acid synthetic peptide. Dual cell surface labelling and cell cycle analysis by FACS revealed that both $CD4^+$ and $CD8^+$ T cells were stimulated in these cultures. The fact that we were able to obtain proliferative responses and interferon-γ (IFN-γ) production to MOMP using cells from cord bloods confirmed that these are genuine primary responses. These experiments have identified a region on MOMP, to which T cells from most humans make a primary response, which may be useful in a chlamydial vaccine. The approach is useful for vaccine development in general.

INTRODUCTION

Chlamydia trachomatis, an obligate intracellular bacterium, is a major cause of human ocular and genital infections world-wide. Perhaps the most devastating of these infections are the estimated 500 million cases of trachoma. This disease, caused principally by serovars A–C of *C. trachomatis* can result in conjunctival and corneal scarring, reduction in vision and eventual blindness. Chlamydial genital tract infection is insidious but is a major world-wide cause of pelvic inflammatory disease resulting in tubal infertility and ectopic pregnancy. In the U.S.A. alone these infections are estimated to cost some 4 billion dollars a year.[1]

The scarring sequelae of chlamydial infection are believed to result from multiple exposure leading to persistent antigen presentation and immunopathological damage. This has hampered attempts to develop a successful vaccine: empirical trials in man and studies in primate models using crude whole organisms produced only short-term serovar-specific immunity and some evidence of long-term genus-specific reactions which enhanced disease severity upon reinfection.[2,3] Thus attention has focused on developing a subunit vaccine in which protective responses and immunopathological reactions can be separated.

The principal candidate for a chlamydial subcomponent vaccine is the chlamydial major outer membrane protein (MOMP). This antigenically complex protein of molecular weight 40,000–44,000 constitutes approximately 60% of the total surface protein of the chlamydial elementary body (EB).[4,5] Functionally, MOMP probably serves as a porin, regulating the chlamydial developmental cycle by the passage of small molecules through the outer membrane.[6] MOMP also stabilizes the chlamydial EB by formation of disulphide-linked oligomers within the outer envelope.[7,8]

Comparison of inferred amino acid sequences for MOMP genes from different serovars has revealed four variable regions (VSI–IV) separated by five conserved sequences.[9,10] The antibody response to MOMP appears to be primarily directed against the surface exposed variable regions, I, II or IV. Serovar-specific epitopes are located within VSI or VSII while species- and subspecies-specific epitopes are located in VSIV.[10–12] Importantly, both polyclonal and monoclonal antibodies to these epitopes on MOMP can neutralize chlamydial infectivity *in vitro*[13–15] or *in vivo*.[16]

Little is known about T-cell recognition of MOMP although animal models suggest that protection against chlamydial infection is T-cell dependent.[17] These cells may provide 'help',

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for the production of neutralizing antibodies to protein antigens and may also produce cytokines that are known to inhibit chlamydial growth reversibly.[18] T cells reactive with the same or distinct determinants may also be responsible for immune damage.

MOMP contains a number of predicted T-cell epitopes when analysed by the methods of Rothbard and Taylor[19] or de Lisi and Berzofsky[20] and regions capable of stimulating proliferative murine T cells in a secondary response are beginning to be described.[21,22] Significantly, one region encompassing the non-surface exposed VSIII is reported to elicit T-cell help *in vivo* for the production of antibodies to protective B-cell epitopes.[22] MOMP epitopes for human T cells and epitopes recognized in the primary immune response remain to be defined.

To analyse the human T-cell responses to MOMP we have assayed primary responses *in vitro* using cells from non-sensitized, naive donors. This approach is dependent upon a technique originally described using murine cells[23] but also used recently for measuring human primary proliferative and cytotoxic T-cell responses to HIV antigens,[24] in which T cells are stimulated in 20-μl hanging drop cultures with antigen-presenting cells (APC) rich in dendritic cells (DC) prepulsed with the antigen. Measuring primary responses has the advantage that it allows determinants which elicit responses in naive individuals to be identified. This must be an essential property for any vaccine. In addition, being able to study responses without the overlying complication of a previous infection at an unknown time may be particularly advantageous for analysing T-cell responses in humans.

In this study we have shown that MOMP is a target of the primary T-cell response to chlamydia in many individuals and we resolve one T-cell epitope recognized by the majority of naive human volunteers to a single conserved peptide of 12 amino acids adjacent to VSIII.

MATERIALS AND METHODS

Bloods

Defibrinated peripheral blood was obtained from healthy laboratory personnel. Naive individuals were those with no history of chlamydial infection, no serum antibodies to *Chlamydia* and no T-cell reactivity to chlamydial antigen in a standard assay of recall responses. Serology was kindly performed by Dr J. Treharne (Institute of Ophthalmology, London, U.K.) using a microimmunofluorescence test which detects IgG, IgM or IgA antibodies to any serovar of *C. trachomatis* or to *C. psittaci* or *C. pneumoniae*. Recall responses to chlamydial antigen were assayed by culturing unfractionated peripheral blood mononuclear cells (PBMC) with an optimal concentration of antigen and measuring proliferation after 5 days (see below). Heparinized (20 U/ml) cord bloods were freshly obtained from the Maternity Unit (Northwick Park Hospital, Northwick, U.K.).

Cell isolation

Mononuclear cells were separated from peripheral or cord bloods by centrifugation over Ficoll–Hypaque (600 ***g***, 25 min) and washed twice in a wash medium of RPMI-1640 containing 2% foetal calf serum (FCS) 100 μg/ml L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. For isolation of rich low density cells (LDC), mononuclear cells were cultured overnight in complete medium in a humidified incubator at 37°. Complete medium was as wash medium except that the Dutch modification of RPMI-1640 (Gibco, Paisley, U.K.) was used and the FCS concentration increased to 10%. Cells were cultured at 4×10^6/ml in T25 tissue culture flasks (Falcon, Cockeysville, MD). After overnight incubation non-adherent cells were centrifuged over metrizamide (made as 14·5 ***g*** plus 100 ml complete medium) at 600 ***g*** for 10 min. LDC were recovered from the interface and washed twice. Pelleted cells were also collected, washed twice and used as a source of lymphocytes. In some experiments T cells were further purified by rosetting with AET-treated sheep red blood cells.[25]

As a source of macrophages plastic-adherent cells from the overnight culture were recovered with a cell scraper (Costar, Cambridge, MA) after incubation on ice for 60 min to aid detachment.

Chlamydial antigens

Chlamydia trachomatis L1/440/LN were grown in BGMK cells in Dulbecco's MEM containing 10% FCS, 100 μg/ml L-glutamine, 200 μg/ml gentamycin and 1 μg/ml cycloheximide. Chlamydial EB harvested after 48 hr culture were partially purified by centrifugation through 25% vol/vol Isopaque (80,000 ***g***, 4°, 60 min). When used as antigen *in vitro*, EB suspensions were irradiated with 1000 Gy from a ^{60}Co source. Recombinant polypeptides comprising the carboxy-terminal 25%, 50% and 75% portions of serovar L1 MOMP were produced in *Escherichia coli* and purified as described previously.[27] Stock preparations were stored at 4° until use.

Peptides were synthesized on Rapid Amide resin on a RAMP (Dupont, Stevenage, U.K.) manual synthesizer by fmoc chemistry in accordance with the manufacturer's instructions. Peptide purity was confirmed by analytical HPLC and by quantitative amino acid analysis. Full details of the selection, synthesis and purification of these peptides have been published elsewhere.[28]

Assay of primary T-cell proliferative responses

Between 5×10^5 and 2×10^6 LDC were pulsed with antigen for 2 hr at 37° in 0·1 ml of complete medium. In preliminary experiments 75% MOMP was used at 1, 10, 100 and 250 μg/ml. For comparison of responses to different-sized fragments, molar equivalent concentrations were used. LDC were pulsed with purified whole L1 EB at a dilution of 1:100 of the irradiated stock preparation (equivalent to approximately 10 μg/ml by Lowry). Pulsed LDC were washed twice, recounted and adjusted to give appropriate cell concentration. Triplicate 20 μl hanging drop cultures were established containing 12,500, 25,000, 50,000 or 100,000 lymphocytes or purified T cells and 250, 500, 1000, 2000 or 4000 LDC which had been antigen pulsed or cultured in medium alone. Cultures were also established to which no LDC were added in order that background turnover could be established. Cultures were incubated for 4 days at 37° in a humidified atmosphere of 5% CO_2 in air. At the end of this period cultures were pulsed for 2 hr with 1 μl (equivalent to 1 μg/ml of thymidine at a specific activity of 2 Ci/mmol) [^{3}H]thymidine and then harvested by blotting onto filter papers.[29] This technique produces low actual counts but stimulation indices are high. Freely available thymidine of low specific activity ensures that the counts reflect DNA synthesis and not availability of precursor or radiation damage.[29] [^{3}H]thymidine incorporation was measured by stand-

ard liquid scintillation counting. The length of culture was varied in some experiments, and in others adherent macrophages were substituted for LDC.

Differences between responses stimulated by control LDC and antigen-pulsed LDC greater than replicate variation were determined by analysis of variance.[30] P-values $<0{\cdot}05$ were regarded as significant.

Primary interferon-γ (IFN-γ) production

Multiple hanging drop cultures containing 100,000 lymphocytes (or purified T cells alone), lymphocytes plus 1500 control LDC or lymphocytes plus 1500 antigen pulsed LDC were established. Drops were harvested after 2, 4 or 6 days of culture and cells removed by centrifugation. Supernatants were stored at -20° until required. IFN-γ was assayed in supernatants using a commercial ELISA kit (Holland Biotechnology, Leiden, The Netherlands) following the manufacturer's instructions. IFN-γ concentration in supernatants was measured in U/ml with reference to a standard curve constructed in parallel.

Cell cycle FACS analysis

To determine the phenotype of T cells responding in a primary response dual cell surface labelling and cell cycle analysis was performed on the FACS.

Cultures were established as described for the IFN-γ experiments. Cells were collected from replicate cultures and labelled with αCD3–fluorescein isothiocyanate (FITC), αCD4–FITC or αCD8–FITC (Becton Dickinson, Mountain View, CA). $7{\cdot}5 \times 10^5$ cells were labelled on ice for 60 min. Control cells were unlabelled. After washing in cold medium cells were permeabilized by the addition of ice-cold 70% ethanol and incubated for 30 min on ice. After washing, the cells were incubated at 37° for 30 min in PBS containing 50 μg/ml propidium iodide (PI) and 100 μg/ml RNase. After further washes the cells were resuspended in phosphate-buffered saline (PBS) containing 2% FCS, 100 mM EDTA, sodium azide and immediately analysed on the FACS. Passage through a 21-g needle was used immediately prior to analysis in order to dissociate any cell doublets. Fluorescence was measured in arbitrary units (AU).

RESULTS

Dose-dependent primary T-cell proliferation stimulated by MOMP fragments

In a pilot experiment, LDC from a non-sensitized donor (no history of infection, no serum antibodies and no recall T-cell response) were pulsed with molar equivalent concentrations of 75%, 50%, or 25% length MOMP (2·7 mM) or with irradiated whole *C. trachomatis* L1 elementary bodies (1 : 100 dilution of a stock preparation) and various numbers used to stimulate autologous T cells in a hanging drop culture system. It can be seen from Fig. 1 that all the chlamydial antigens stimulated a significant proliferative response with stimulation indices reaching eight times background for each antigen. The response to the three MOMP fragments was of similar magnitude but the response to whole EB was weaker particularly at lower LDC concentrations. At high LDC numbers (4000/well) antigen-specific responses were partially masked by high levels of stimulation by autologous LDC not pulsed with antigen (autologous mixed leucocyte reactivity). These data underlie the need to test responses across a range of LDC numbers.

In a second experiment reactivity of this donor's T cells to 75% MOMP was confirmed and the response shown to be antigen dose dependent (data not shown). Maximal responses for MOMP were obtained at a pulsing concentration of 2·7 mM (100 μg/ml 75% MOMP). The response was reduced but still significant at 0·27 mM and not detectable at 0·027 mM. In an additional experiment, the pulsing concentration of 75% MOMP was increased to 6·75 mM, the highest practicable concentration, but the proliferative response was not distinguishable from that obtained with 2·7 mM (data not shown).

Primary responses to chlamydial antigens are detectable with T cells from most naive individuals

In the next stage of the study, four more non-sensitized individuals and one with evidence of sensitization to chlamydial antigen were studied (Table 1). All but one donor responded to one or more of the chlamydial antigens tested but the pattern of response varied between individuals. Donor 2 displayed a pattern of reactivity similar to donor 1 (described in the previous

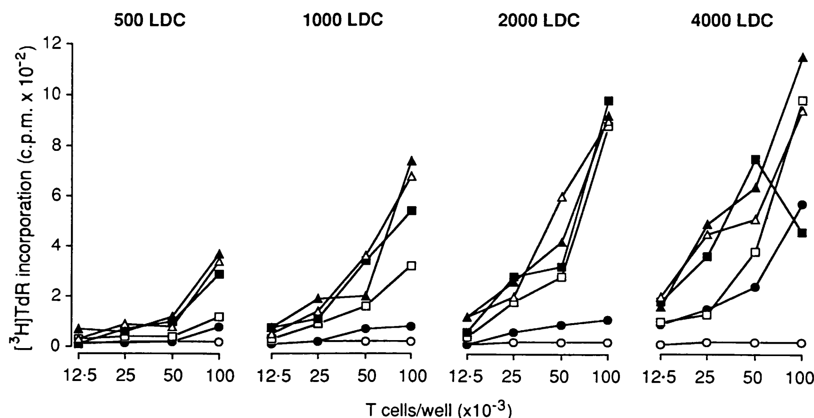

Figure 1. Primary T-cell proliferative responses to chlamydial antigens. Purified T cells from a naive donor were cultured alone (○), or with 500, 1000, 2000 or 4000 autologous LDC. LDC were unpulsed with antigen (●) or pulsed with 2·7 mM 25% MOMP (■), 50% MOMP (△), 75% MOMP (▲) or L1 EB at 1 : 100 dilution (□). Proliferation was measured on day 4 of culture.

Table 1. Primary T-cell responses to chlamydial antigens are detectable in most individuals

Donor	2° response	1° response				HLA type
		EB	75% MOMP	50% MOMP	25% MOMP	
1	–	+	+	+	+	A2,3,36 B58 41 Bw4,6, Cw2,3 DR7, w53 DQ2
2	–	+	+	+	+	A1,2 B8,44 w4,6 Cw5,7 DR1,3 w52 DQ1,2
3	–	ND	–	ND	ND	A3,10,26 B7,12 w4 w6 Cw5,w7 DR2,5w52 DQ1,3
4	ND	+	–	–	–	A3 w29 w19 B12,35 DR1
5	–	–	+	+	+	A2 B13,44 Cw4 w6 DR2,7 DQ1,2
6	+	ND	+	ND	ND	A1,32 B7,8 w6 Cw7 DR2,3 w52 DQ1,2

ND, not done.

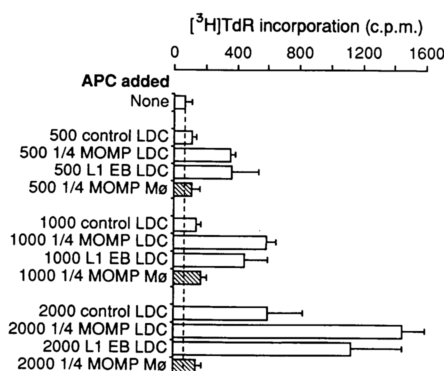

Figure 2. Only APC preparations containing DC are able to stimulate primary responses. Purified T cells were cultured at 100,000/well alone or with 500, 1000 or 2000 APC. These were DC-rich LDC unpulsed with antigen, pulsed with 25% MOMP or pulsed with L1 EB. Alternatively they were adherent macrophages (Mϕ) pulsed with 25% MOMP. There were insufficient Mϕ to test EB. Proliferation was assayed on day 4.

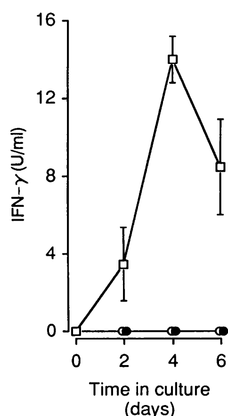

Figure 3. Kinetics of primary IFN-γ production. Replicate cultures containing 100,000 lymphocytes alone (○) or stimulated with 1500 control LDC (●) or 1500 75% MOMP-pulsed LDC (□) were established. Supernatants were harvested at the times indicated and IFN-γ assayed as described in Materials and Methods. $t = 0$ days, complete medium alone.

section). Donor 4 responded to the whole bacterial antigen but not to the MOMP fragments whereas donor 5 displayed the reciprocal pattern. APC from donor 4 may process EB in a way that does not yield appropriate MOMP T-cell epitopes. We were only able to test donor 3 for reactivity to 75% MOMP but no response was found. Donor 6 recognized 75% MOMP and we had evidence of prior sensitization in this donor, indicating that MOMP is also a target for a secondary T-cell response.

Dendritic cells are required for primary response to MOMP and whole EB

To determine whether DC were required for primary responses to chlamydial antigens we compared the ability of LDC (approximately 30% DC with the majority of the remaining cells being low density monocytes) and adherent macrophages to stimulate a primary response to EB and to 25% MOMP. These were respectively the most complex and simplest of the antigens we had studied to date. As shown in Fig. 2, LDC but not macrophages were able to stimulate responses to both antigens. This indicates that DC are required in order to obtain a primary response although a contribution by the monocytes contaminating the LDC population, perhaps in processing the EB, cannot be excluded.

Primary IFN-γ production

We looked for the presence of IFN-γ in hanging drops after 2, 4 or 6 days of primary culture. Lymphocytes were cultured on their own, with control LDC or with LDC pulsed with 75% MOMP. IFN-γ was detected only in cultures stimulated with 75% MOMP (Fig. 3). The lymphokine was detectable after 2 days in culture (the first time-point tested) and maximal levels were reached after 4 days. The maximal level of concentration reached in this experiment was 14 U/ml but in further experiments very high levels (> 30 U/ml) have been detected (data not shown).

Both CD4$^+$ and CD8$^+$ T cells are stimulated in a primary response to chlamydial antigen

In order to phenotype the cells responding in a primary *in vitro* response to MOMP, surface labelling and cell cycle analysis on the FACS were performed. The data in Fig. 4 are from one experiment, representative of the two performed. Consistent

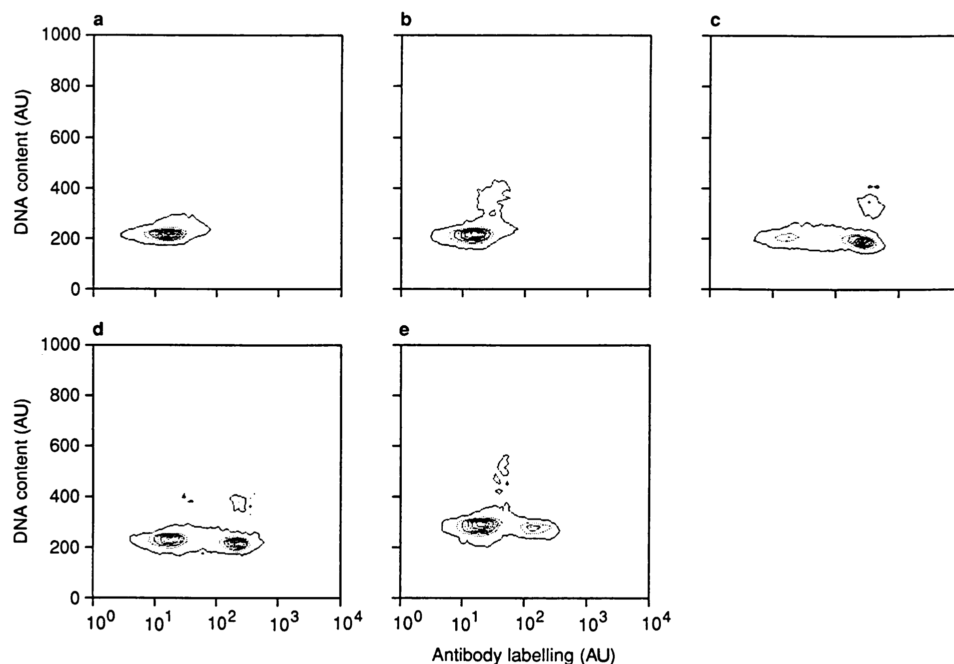

Figure 4. Dual surface labelling and cycle analysis of cells responding in a primary response. (a) Lymphocytes cultured with 2000 control LDC; PI labelling only. (b) Lymphocytes cultured with 2000 75% MOMP-pulsed LDC; PI labelling only. (c) As (b) but dual labelled with anti-CD3–FITC. (d) As (b) but dual labelled with anti-CD4–FITC. (e) As (b) but dual labelled with anti-CD8–FITC.

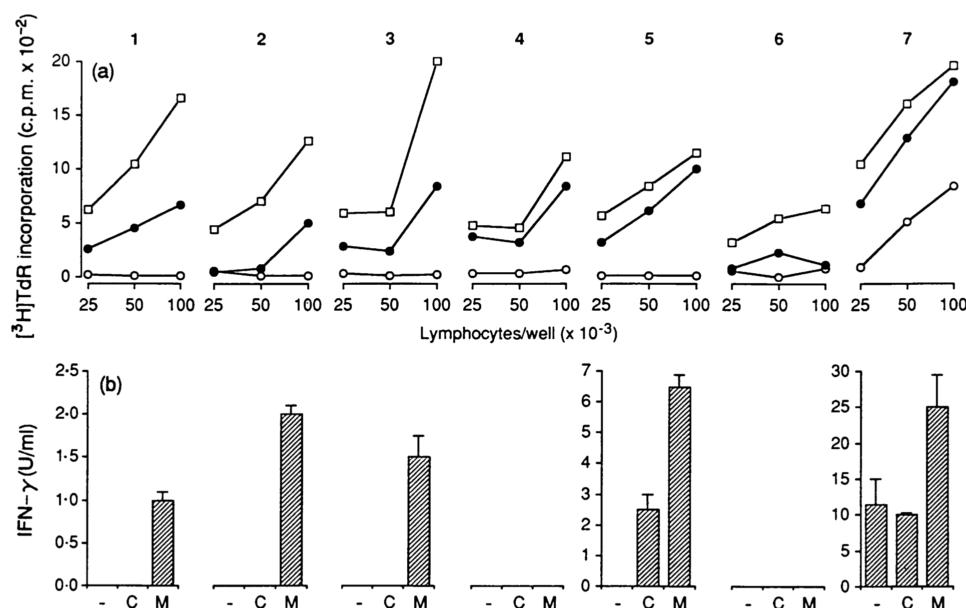

Figure 5. Primary proliferation (a) and IFN-γ production (b) by cells from seven cord bloods. (a) Lymphocytes alone (○); lymphocytes cultured with control LDC (●); lymphocytes stimulated with 75% MOMP pulsed LDC (□). Purified T cells were used from cords 1–5 and crude lymphocytes from cords 6 and 7. (b) Supernatants from lymphocytes alone (-), lymphocytes plus control LDC (C); or lymphocytes plus 75% MOMP-pulsed LDC (M). Cells used as for (a).

with the proliferation and IFN-γ data, cells in cycle were detectable in cultures stimulated with 75% MOMP pulsed LDC but not in those stimulated by control LDC (Fig. 4b versus 4a). In these experiments the DNA content of cells in G0 and G1 (resting cells) was approximately 200 AU as determined by PI labelling. Cells in G2 and M had a DNA content of approximately 400 AU and cells in S phase had intermediate values. 4·93% of the cells were in cycle following stimulation with antigen-pulsed LDC compared with 0·15% in the control cultures. Almost all of the cells in cycle were labelled with anti-CD3 (Fig. 4c) indicating that they were T cells. 4·47% of the cells were $CD3^+$ and in cycle.

Dual labelling with anti-CD4 revealed that the majority of cells in cycle were $CD4^+$ although a $CD4^-$ population was also

detectable (Fig. 4d). 2·99% of the cells were CD4+ and in cycle, and 1·19% were CD4− and in cycle.

It appears that the CD4− cycling cells are probably CD8+ T cells. This is not apparent on the printout shown in Fig. 4e, dual labelling with CD8, where only the predominant CD8− population is readily seen. However, statistical analysis revealed that 2·77% of the cells were CD8+ and in cycle. This compares with 0·02% in the equivalent labelling of lymphocytes stimulated by control LDC.

Thus both CD4+ and CD8+ T cells are activated in the primary response.

Primary responses of T cells from cord bloods

It could be argued that despite our efforts to exclude sensitized individuals from our panel of healthy donors, the responses we obtained could be attributed to low levels of prior sensitization to *Chlamydia* not detectable in our assays, or to cross-reactivities with other antigens to which the donors had been exposed. We therefore sought to confirm the observations using a truly naive cell population isolated from cord bloods. We looked at both proliferation and IFN-γ production stimulated by 75% MOMP. Preliminary experiments established that the response kinetics were simiar to those with cells from PB with peak responses occurring on day 4 (not shown). Figure 5a shows the proliferation data, and Fig. 5b the IFN-γ production for the first seven cord bloods studied.

As before, proliferation was assayed across a range of LDC concentrations, although for clarity only the optimal responses are shown in Fig. 5a. Significant antigen-specific proliferation was obtained with three of seven cord bloods (cords 1, 2 and 3). In some of the remaining four there are possibly small responses to 75% MOMP but these did not attain statistical significance. Antigen-driven IFN-γ production was detectable with cells from these three cords and in two of the others (5 and 7) although the absolute level of IFN-γ present in the cultures varied considerably. Only with cells from one cord (cord 5) did stimulation by control LDC result in release of IFN-γ above that observed in cultures of lymphocytes alone.

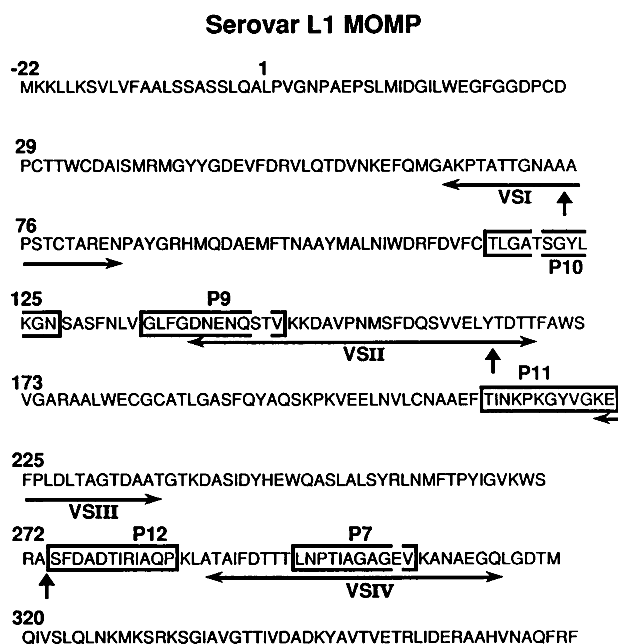

Figure 6. Predicted amino sequence of serovar L1 MOMP. Boxed regions show location of synthetic peptides studied. Variable regions VSI–IV are shown underlined. Breaks in boxed sequence indicate residues at which tested peptides, from different serovars, differed from the L1 sequence.

Primary T-cell responses to synthetic MOMP peptides

To begin to map the fine specificity of the primary T-cell response to MOMP we screened a small panel of synthetic peptides from the MOMP sequence, which were available in the laboratory, for their ability to stimulate responses in normal donors. The location of the peptides (11–12 amino acids plus an addition carboxy terminal cysteine) in the L1 sequence are shown in Fig. 6. All four of the five peptides containing predicted T-cell epitopes stimulated responses from some donors (Table 2). Peptide 7 (P7) which was not predicted to contain an epitope failed to stimulate cells from all four donors tested. Peptide 11 (P11) lying close to VSIII, stimulated the highest proportion of responses (80%). For example, maximal thymidine incorporation by donor T cells stimulated by P11-pulsed LDC was 472 c.p.m. compared to 85 c.p.m. when the T cells were stimulated with LDC exposed to vehicle only. It is

Table 2. Primary T-cell responses to MOMP peptides

Peptide	Predicted* T-cell epitope	Response of donor† 1	2	3	4	5	6	7	8	9	10	11	12	13	Total
P7	–	–	–	–	–										0/4
P9	B							–	+	–					1/3
P10	B/R	–	+	–	–										1/4
P11	B/R	–	+	+		+	+	–	+	+	+			+	8/10
P12	B/R		+	–				+							2/3

* Predicted by the methods of Berzofsky (B) or Rothbard (R). See text for references.

† (+) Responses to peptide pulsed LDC were significantly ($P < 0{\cdot}05$) greater than that to LDC pulsed with vehicle alone; (–) no significant response; gap, not tested.

notable that the response of a given donor is often not restricted to a single peptide.

DISCUSSION

In this study we have shown that it is possible to assay primary human T-cell proliferation and IFN-γ production to the candidate chlamydial vaccine antigen MOMP. Using a similar technique, previous work from this laboratory[24] has described primary proliferative responses to human immunodeficiency virus (HIV). However this report represents the first description of primary lymphokine production and identifies the phenotype of the cells responding.

MOMP-pulsed LDC were able to stimulate proliferative and IFN-γ responses from cells from a large proportion of both healthy controls, PB and cord blood. Our interpretation is that these responses are primary T-cell responses by naive T cells but at least three other possibilities need to be considered. Firstly, the possibility that our donors were in fact sensitized to chlamydial antigens but we were unable to detect this in the assays employed. It is known, for instance, that exposure to the respiratory pathogen *C. pneumoniae* is widespread.[26] Such exposure would not explain why we were able to obtain responses using cord blood cells which comprise $>95\%$ phenotypically naive T cells.[31] Furthermore, only cell preparations containing DC were able to stimulate the responses we observed but macrophages are able to act as APC for recall responses (A. J. Stagg, W. A. J. Elsley, M. A. Pickett, M. E. Ward and S. C. Knight, unpublished observation). Such data also argue against the possibilities that the observed responses are due to cross-reactions between MOMP and non-chlamydial antigens to which the donors were sensitized or to the reactivity being directed against trace *E. coli* derived contaminants in the recombinant protein preparations. The observation that responses could also be obtained with a synthetic peptide derived from the MOMP sequence also argues against the latter possibility. Furthermore the dependence on DC to stimulate a response would argue against activation of T cells by a non-specific pathway independent of major histocompatibility complex (MHC)-restricted antigen presentation.

The evidence presented here indicates the presence of several major epitopes for human T cells in MOMP. In particular, one which stimulated T cells from approximately 80% of normal donors has been mapped to a 12 mer peptide (peptide 11) lying just before VSIII. The algorithms of both de Lisi and Berzofsky[20] and Rothbard and Taylor[19] predict a large number of potential T-cell epitopes in MOMP and experimental studies in the mouse have identified a number of regions capable of stimulating T-cell proliferation. Su *et al.*[21] reported that eight of 25 overlapping peptides covering the entire *C. trachomatis* serovar A MOMP primary sequence were capable of stimulating spleen cells from A/J mice primed with MOMP. Using a slightly different approach Allen *et al.*[22] found that five of nine lac Z fusion proteins covering the MOMP sequence stimulated spleen cells from BALB/c mice inoculated with viable organisms. Six of the fusion proteins were able to prime mice for *in vitro* reactivity to whole organisms. However, in contrast to the proliferation data, only one peptide, a fusion peptide of 61 amino acids and encompassing VSIII was found to be capable in a functional assay of eliciting T-cell help *in vivo* for the production of antibodies to protective B-cell epitopes. The assay used involved priming with the test fusion peptide then assaying MOMP-specific Ig following boosting with whole non-viable organisms. More Th epitopes were reported in the earlier study of Su *et al.*[21] who used A/J mice and boosted with MOMP instead of whole organisms but again it was possible to identify at least one peptide which stimulated secondary T-cell proliferation but which did not elicit help for antibody production, indicating that these two functions are dissociated.

Peptide 11 which we have identified as being able to stimulate primary T-cell proliferation in the majority of human donors lies within the MOMP fusion peptide reported by Allen *et al.*[22] which stimulated both proliferation and elicited help in BALB/c mice. However, this peptide is in a region which failed to stimulate A/J splenic T-cell proliferation in the study of Su *et al.*[21] Fine mapping of the epitope with peptide 11 has yet to be performed but since only the two carboxy terminal amino acids lie within VSIII the epitope is likely to be conserved between serovars. This, coupled with its ability to stimulate T cells from the majority of individuals, makes it an attractive candidate vaccine component. The implication of our findings is that peptide 11 can be recognized in the context of many different MHC class II molecules although this remains to be formally tested. Sinigaglia *et al.*[32] have described a peptide from the *Plasmodium falciparum* circumsporozoite protein that is recognized in association with most mouse and human MHC class II molecules. It now becomes important to study the ability of peptide 11 to elicit T-cell help and the production of lymphokines that may be important in defence against intracellular infection, as distinct from others which may mediate immunopathology.

Stimulation with MOMP-pulsed LDC also led to the primary production of IFN-γ in culture. This lymphokine reversibly inhibits chlamydial growth *in vivo*[33] and *in vitro*[34] and may thus be an important aspect of defence against *Chlamydia*. It is notable that IFN-γ production was detectable in some cultures where there was no proliferation, indicating that these two responses may be separable with distinct signalling pathways or, perhaps, IFN-γ production by an additional cell type. In the mouse, helper T cells have been divided into two major subsets based upon the analysis of lymphokine production by panels of T-cell clones (reviewed in ref. 35). Th1 cells produce interleukin-2 (IL-2) and IFN-γ and mediate delayed-type hypersensitivity (DTH) responses whereas Th2 cells produce IL-4 and may be regarded as classical helper T cells for antibody production. To date, it is unclear whether such a clear dichotomy exists for human T cells but there is evidence that the human immune response may be biased towards a Th1 or Th2 type. If analogies can be drawn with the murine system, we have demonstrated primary activation of the Th1 pathway and we are currently studying the production of lymphokines associated with Th2 cells. Of particular interest are IL-5 and IL-6. IL-5 in murine systems at least, acts synergistically with transforming growth factor-β (TGFβ) (a product of several cell types including T cells) to enhance B-cell isotype switching toward the production of IgA.[37] Secretory IgA directed against surface-exposed chlamydial epitopes may be an important defence mechanism for preventing the establishment of initial chlamydial infection. The Th2 product IL-6 may be involved in scarring immunopathology. It could be argued that stimulation of a Th1-type response may be detrimental to the host due to the initiation of DTH and immunopathology.[38] However, recent

animal studies in the guinea-pig have found that a 57,000 MW heat shock protein but not MOMP is able to stimulate damaging DTH reactivity in chlamydia-sensitized animals.[39] Clearly this is an area which requires further study.

Our cell cycle analysis data demonstrate that the primary response to MOMP involves both CD4+ and CD8+ T cells. Preliminaty experiments have also indicated blocking of the proliferative response with anti-MHC class II antibodies but not anti-MHC class I (data not shown). It is not yet clear whether activation of CD8+ T cells represents 'bystander' activation or genuine antigen-specific stimulation of class I-restricted T cells in the cultures. DC are potent accessory cells for the generation of CD8+ cytolytic T lymphocytes (CTL)[40] and there is evidence that exogenous soluble antigens can gain access to the class I MHC-restricted pathway of antigen presentation.[41] Furthermore primary CTL have been generated to HIV antigens *in vitro*[24] using a culture method identical to that described here. We are currently looking for Chlamydia-specific CTL in our cultures.

In conclusion, we have demonstrated the feasibility of measuring primary human T-cell responses to chlamydial antigens and have shown that this technique can be used for mapping target epitopes and the type of lymphokine response they stimulate. This approach should prove very useful in the development of a chlamydial vaccine.

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