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Intracellular processing and presentation of T cell epitopes, expressed by recombinant *Escherichia coli* and *Salmonella typhimurium*, to human T cells*

Vaccines based on recombinant attenuated bacteria represent a potentially safe and effective immunization strategy. A carrier system was developed to analyze *in vitro* whether foreign T cell epitopes, inserted in the outer membrane protein PhoE of *Escherichia coli* and expressed by recombinant bacteria, are efficiently processed and presented via human leukocyte antigen (HLA) class I and II molecules by bacterial infected human macrophages. A well-defined HLA-B27-restricted cytotoxic T cell (CTL) epitope and an HLA-DR53 restricted T helper (T_h) epitope of the fusion protein of measles virus were genetically inserted in a surface-exposed region of PhoE, and the chimeric proteins were expressed in *E. coli* and *Salmonella typhimurium*. Macrophages infected with both recombinant bacteria presented the T_h epitope to the specific CD4⁺ T cell clone, but failed to present the CTL epitope to the specific CD8⁺ T cell clone. Presentation of the T_h epitope by the infected macrophages was inhibited by cytochalasin D, indicating that phagocytic processing of intact bacteria within infected macrophages was essential for antigen presentation via HLA class II. Presentation of the T_h epitope to the CD4⁺ T cell clone by infected macrophages was blocked by brefeldin A and cycloheximide, indicating the requirement of nascent HLA class II molecules for presentation. The efficiency of macrophages to process and present the inserted T_h epitope was similar for both the recombinant *E. coli* and *S. typhimurium* strains.

1 Introduction

Avirulent *Salmonella* strains have been generated to be used as live *Salmonella* vaccine [1]. These attenuated *Salmonella* strains and several *E. coli* strains have also been evaluated for their ability to present heterologous antigens to the immune system [2–8]. In such recombinant strains bacterial carrier proteins are frequently employed to present inserted antigenic determinants in the appropriate immunogenic form to the immune system of the host.

While the T cell-mediated immune response to intracellularly residing bacteria was initially thought to be solely mediated by CD4⁺ T cells, the additional role of CD8⁺ T cells is now generally accepted [9]. The role of CD8⁺ T cell-mediated immunity against intracellular bacteria has first been shown for *Listeria monocytogenes* [10, 11]. Since *L. monocytogenes* enters the cytosol after lysis of the phagosome, the peptides presented by MHC class I mole-

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cules were originally thought to be generated from degradation of bacterial proteins in the cytosol [12]. However, it has recently been shown that listerial antigens can also be introduced into the MHC class I pathway independent of the egression of *L. monocytogenes* from the phagosome [13]. The ability of *Mycobacterium* [14, 15] and *Salmonella* [16, 17], which reside intracellularly within phagosomes, to elicit specific CD8⁺ T cell responses *in vivo*, underlines the possibility that antigens located within the endosomal compartment can be presented by MHC class I molecules. Moreover, oral immunization of mice with attenuated *S. typhimurium* strains expressing heterologous antigens was shown to induce a specific CD8⁺ T cell response [18], which in some cases conferred protective immunity to the specific pathogen [8]. The processing pathway for presentation of T cell epitopes expressed by the phagosomally residing *E. coli* and *S. typhimurium* has recently been studied *in vitro* using peritoneal mouse macrophages [19, 20]. It was observed that the T cell epitopes were efficiently processed and presented via MHC class I and II. In contrast, other groups have not been able to show class I presentation *in vitro* of recombinant bacterial antigens by cells infected with *S. typhimurium* [7, 21].

We have previously shown that both B- and T-cell epitopes can be inserted within the surface-exposed regions of the *E. coli* outer membrane protein PhoE without disturbing the biogenesis of the protein (for a review see [22]). Each monomer of this trimeric protein traverses the outer membrane 16 times in an antiparallel β-sheet structure, thereby exposing eight regions at the cell surface [23]. In the rat system, using purified chimeric PhoE trimers, CD4⁺ T cell responses specific to an inserted epitope have been observed *in vitro* and *in vivo* [24, 25].

In the present study, an *in vitro* model system was developed to analyze the applicability of the PhoE carrier protein to permit presentation of inserted T cell epitopes via HLA class I and II by human macrophages infected with recombinant bacteria. Well-defined CTL and T_h epitopes of measles virus were employed and the efficacy of recombinant *E. coli* and an attenuated *S. typhimurium* strain to present the epitopes were compared.

2 Materials and methods

2.1 Bacterial strains and growth conditions

The *E. coli* K-12 strain CE1224 produces no pore proteins due to mutations in the *phoE* and *ompR* genes [26]. The *S. typhimurium* strain SL3261, obtained from B. A. D. Stocker (Stanford University, CA), is unable to synthesize aromatic amino acids and is therefore avirulent, but it has not lost its ability to enter (non-) phagocytic cells [1]. Bacteria were grown overnight at 37 °C under aeration in Luria-Bertani (LB) medium, supplemented where necessary with ampicillin (50 µg/ml). Bacteria were inactivated by heat treatment for 15 min at 100 °C.

2.2 MVF peptides and T cell clones

Synthetic peptides corresponding to the T cell epitopes of MVF, peptide 437–447 (CTL epitope) and peptide 453–464 (T_h epitope), were prepared by solid-phase synthesis [27]. The isolation, maintenance and properties of the MVF-specific CD4$^+$ (JP-F94) and CD8$^+$ (WH-F40) T cell clones used have been described [27]. The T cell clones JP-F94 and WH-F40 recognize the epitopes PPISLERLDVGT (amino acid residues 453–464) and SRRYPDAVYLH (residues 437–447) in association with HLA-DR53 and HLA-B27, respectively [27].

2.3 Construction and characterization of the chimeric PhoE proteins

Chimeric proteins consisting of the *E. coli* PhoE protein with insertions of the T_h or CTL epitope of MVF were genetically constructed by the strategy described previously [24]. Restriction enzymes and other DNA modifying enzymes were purchased from Pharmacia (Uppsala, Sweden). The expression vector pMR08 contains the *phoE* gene with a Bam HI linker insertion [28]. PMR08 was digested with Bam HI and ligated with T4 DNA ligase to the linkers encoding the T cell epitopes (Fig. 1). Insertion in the wrong orientation would result in the introduction of a stop codon in the reading frame of PhoE. Correct insertion of the linkers was verified by sequencing using the T7 DNA polymerase sequencing kit (Pharmacia, Uppsala, Sweden). The plasmids containing the CTL- and T_h epitope were designated pRM30 and pRM31, respectively. Plasmid pRM01 contains the wild-type *phoE* gene behind the *lac* promoter [29]. The recombinant *phoE* genes were inserted behind the *lac* promoter by digestion of pRM30, pRM31 and pRM01 with MluI and BglII and ligation of the appropriate DNA fragments with T4 DNA ligase. The resulting plasmids encoding PhoE with the inserted CTL and T_h epitopes were designated pRM40 and pRM41, respectively.

2.4 Human macrophages

Tissue culture reagents were obtained from Gibco Laboratories (Grand Island, NY). Cells were cultured in complete medium: RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS-c), penicillin (100 IU/ml) and streptomycin (100 µg/ml). PBMC were isolated from heparinized blood samples of healthy HLA-matched blood donors by Ficoll-Paque density gradient centrifugation (Pharmacia, Uppsala, Sweden). PBMC were depleted of monocytes by differential adherence to plastic petridishes. The nonadherent cells were removed with pre-warmed (37 °C) complete medium and the adherent cells were harvested with a rubber policeman and ice-cold phosphate-buffered saline (PBS) [30]. The adherent cells, ~95% monocytes, were seeded at a concentration of 2×10^4 per well (100 µl) in 96-well round-bottom microtiter plates. To obtain macrophages, monocytes were cultured for 14 days at 37 °C in a CO_2 incubator. During this period, the monocytes had acquired a macrophage morphology and function [31].

2.5 T cell proliferation assays

HLA-matched macrophages were used as antigen-presenting cells. Macrophages were incubated in RPMI 1640 medium containing 10% heat-inactivated pooled human serum (HS-c) with various amounts of peptide, viable or heat-killed recombinant bacteria for 2 h at 37 °C in a CO_2 incubator. Following incubation, the macrophages were extensively washed with RPMI 1640 and incubated for 1 h at 37 °C in a CO_2 incubator in medium containing 200 µg/ml gentamycin to kill extracellular bacteria [32]. The medium was replaced by medium containing 20 µg/ml gentamycin and the cells were irradiated (300 rad). The T cell clones JP-F94 (2×10^4 cells/well) or WH-F40 (5×10^4 cells/well) were added to the macrophages and cultured for 3 days at 37 °C in a CO_2 incubator. The cells were labeled with [^{3}H] thymidine during the last 18 h of culturing, harvested, and the incorporated radioactivity was counted in a flat-bed β-scintillation counter.

2.6 Treatment of macrophages with drugs

To determine the kinetics and requirements for antigen processing, the macrophages were fixed for 30 s with 0.05% glutaraldehyde [21], either before or at different time points after incubation with the antigen preparations. The reaction was stopped by adding L-glycine at a final concentration of 200 mM, and the macrophages were washed several times with RPMI 1640 containing 20 mM L-glycine. Subsequently, RPMI 1640 supplemented with 10% HS-c and 20 µg/ml gentamycin was added and the fixed macrophages were used as stimulator cells in proliferation assays. If appropriate, the macrophages were pretreated for 30 min with brefeldin A (1 µg/ml), cycloheximide (10 µg/ml) or cytochalasin D (5 µg/ml) (Sigma, St. Louis, MO). In the presence of the same concentrations of inhibitors, macrophages were incubated with the antigen preparations for 2 h, washed, and reincubated for 3 h as described in Sect. 2.5. The macrophages were then washed extensively and fixed with glutaraldehyde.

3 Results

3.1 Construction of plasmids and characterization of chimeric PhoE proteins

To study the applicability of the PhoE protein to permit the presentation of inserted epitopes via MHC class I and II in human macrophages, two chimeric PhoE proteins were constructed. Synthetic oligonucleotide linkers coding for the HLA-B27-restricted epitope 437–447 and for the HLA-DR53 restricted epitope 453–464 of MVF were in the DNA encoding the fourth cell-surface exposed region of the PhoE protein (Fig. 1). The recombinant plasmids encoding the chimeric PhoE proteins with either the CTL or the T_h epitope under control of the *lac* promoter, were designated pRM40 and pRM41, and the chimeric PhoE proteins are referred to as PhoE-CTL4 and PhoE-TH4, respectively. These plasmids were used to transform the *E. coli* K-12 strain CE1224 and the attenuated *S. typhimurium* strain SL3261. Analysis of cell envelopes and peptidoglycan-protein complexes by SDS-PAGE and Western immunoblotting revealed that the chimeric PhoE proteins were constitutively expressed at high levels and assembled into trimers (data not shown). This indicates that the chimeric proteins were correctly assembled in the outer membrane.
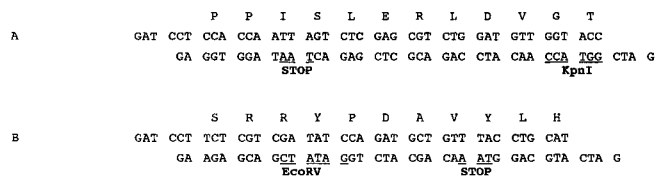

Figure 2. Macrophages infected with recombinant *E. coli* CE1224 and *S. typhimurium* SL3261 bacteria expressing the T_h epitope are recognized by the specific CD4$^+$ T cell clone JP-F94. Macrophages (2.10^4) were incubated with different concentrations of peptide 453–464 (•), CE1224.pRM41 (●) or SL3261.pRM41 (○) which express PhoE-TH4, or CE1224.pRM40 (■) and SL3261.pRM40 (□) which express PhoE-CTL4. The multiplicity of infection (MOI) is indicated. After incubation, the macrophages were washed extensively and proliferation of the T cell clone was measured as described in Sect. 2.5. The results are expressed as mean [^{3}H] thymidine incorporation ± SD of triplicate cultures.

Figure 1. Nucleotide sequences of the linkers, consisting the complementary oligonucleotides, that were used for the insertion of the MVF T cell epitopes in the fourth cell surface-exposed region of PhoE. The amino acid sequences of the CTL (A) and T_h epitopes (B) are indicated above the linker. The indicated restriction enzyme sites and stop codons were included in the oligonucleotides to facilitate screening of recombinant plasmids.

3.2 T cell recognition of human macrophages infected with recombinant bacteria

To determine whether the inserted MVF T_h epitope can correctly be presented via HLA class II, human macrophages were infected with the recombinant strains expressing PhoE-TH4. The infected macrophages were able to process and present the inserted T_h epitope to the specific CD4$^+$ T cell clone JP-F94 (Fig. 2). For both bacterial strains, the optimal T cell response was observed at a multiplicity of infection (MOI) of 10. As expected, the T cell clone also responded to macrophages pulsed with the corresponding synthetic MVF peptide, but not to macrophages infected with the recombinant bacterial strains expressing PhoE-CTL4 (Fig. 2). This indicates that the observed T cell response is not attributable to nonspecific mitogenic effects of bacterial products.

To determine whether the inserted CTL epitope can correctly be presented via HLA class I, macrophages were infected with recombinant bacteria expressing PhoE-
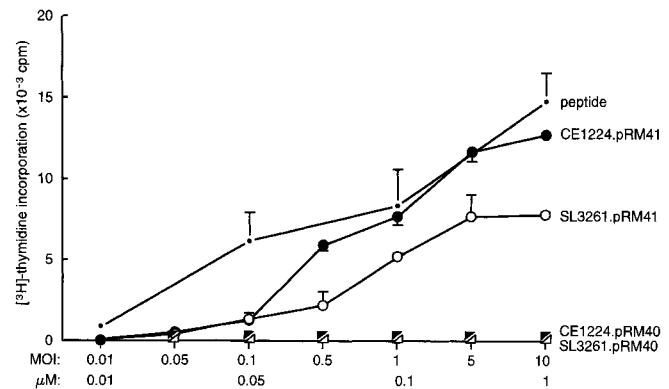
CTL4. In contrast to macrophages pulsed with the corresponding synthetic MVF (437–447) peptide, the infected macrophages were not recognized by the T cell clone WH-F40 (data not shown). The possibility that the bacterial infection disrupted the expression of HLA-B27 or accessory molecules required for proliferation of the T cell clone, was excluded by the observation that the infected macrophages were still able to present the synthetic MVF (437–447) peptide (data not shown).

3.3 Kinetics of antigen processing in human macrophages

To determine the kinetics of antigen processing, macrophages were fixed with glutaraldehyde to stop processing before or at several time points after the addition of either peptide, viable or heat-killed bacteria expressing PhoE-TH4. Prefixation of the macrophages abolished presentation of the T_h epitope by macrophages incubated with either viable or heat-killed recombinant bacteria, but not by those incubated with the MVF peptide (453–464) (Fig. 3). This indicates that processing of PhoE-TH4 by viable macrophages is required for presentation of the T_h epitope. Optimal presentation of the T cell epitope was observed within 1–3 h und 3–5 h for macrophages incubated with heat-killed or viable bacteria, respectively. Maximal T cell recognition of macrophages pulsed with peptide was observed after 1 h of incubation (Fig. 3). No differences were observed between the bacterial strains used.

3.4 Inhibition of presentation of the MVF T_h epitope by cytochalasin D, brefeldin A and cycloheximide

To determine whether the presentation of the T_h epitope by infected macrophages was actually due to phagocytic processing of intact bacteria, rather than to processing of
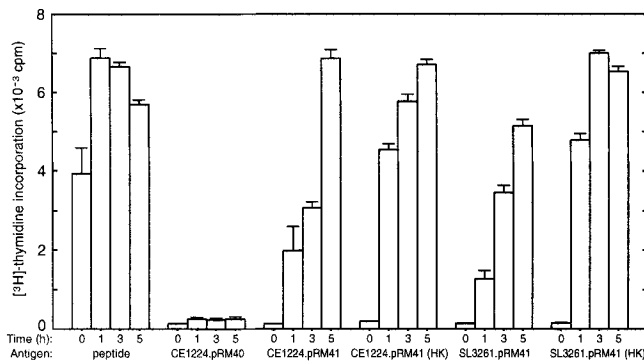
Figure 3. Kinetics of processing of PhoE-TH4 expressed by recombinant *E. coli* CE1224 and *S. typhimurium* SL3261 bacteria by human macrophages for presentation of the T_h epitope. Macrophages were incubated with peptide 453–464 (1 μM), with CE1224.pRM41 or SL3261.pRM41 bacteria expressing PhoE-TH4, or with CE1224.pRM40 expressing PhoE-CTL4. Viable or heat-killed bacteria (HK) were added at an MOI of 10. Cells were fixed with glutaraldehyde before (t = 0 h) or at different time points after antigen exposure. Proliferation of the specific CD4+ T cell clone JP-F94 was measured. The results are expressed as mean [3H] thymidine incorporation ± SD of triplicate cultures.

PhoE-TH4 released from lysed bacteria, cytochalasin D was used. Cytochalasin D inhibits the phagocytic capability of cells [33]. Treatment of macrophages with cytochalasin D before incubation with the viable recombinant bacteria of both strains expressing PhoE-TH4 abolished the proliferative response of the T cell clone JP-F94 (Fig. 4). On the other hand, cytochalasin D-treated macrophages showed optimal T cell stimulation upon incubation with peptide or heat-killed bacteria.

Pretreatment of macrophages with brefeldin A or cycloheximide inhibited the presentation of the T_h epitope after incubation with either viable or heat-killed recombinant bacteria (Fig. 4). No differences were observed between the bacterial strains. In contrast, drug-treated macrophages were still able to present the MVF (453–464) peptide.
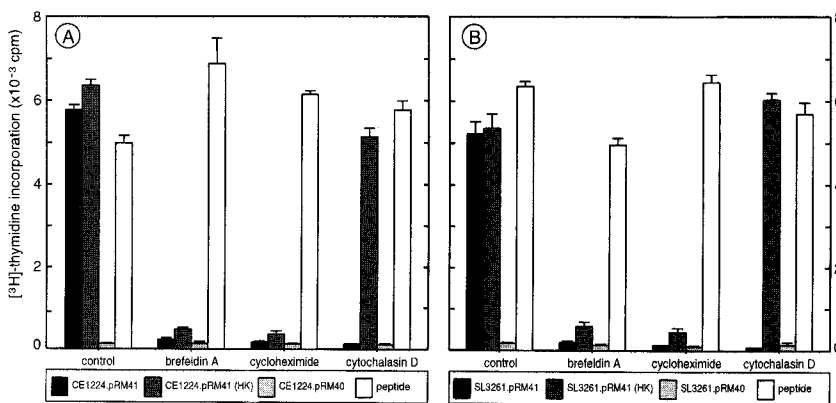
4 Discussion

We have previously shown in the rat system that purified chimeric PhoE proteins containing class II-restricted T cell epitopes are, both *in vitro* and *in vivo*, presented to CD4+ T cells [24, 25]. We have now demonstrated that a T_h epitope expressed as chimeric PhoE protein in *E. coli* and attenuated *S. typhimurium* is efficiently processed and presented to human CD4+ T cells by infected human macrophages.

In contrast to *E. coli* K-12, *S. typhimurium* survives within macrophages by inhibiting the fusion of phagosome and lysosome [34]. Since phagosome/lysosome fusion is required for processing and presentation of internalized bacterial antigens by class II molecules [35], we compared the antigen processing kinetics of PhoE-TH4 in macrophages infected with either the *E. coli* strain CE1224.pRM41 or the *S. typhimurium* strain SL3261.pRM41. Surprisingly, maximal presentation of the inserted epitope was observed within a similar time period for both bacterial strains, *i.e.* 3–5 h after infection. Therefore, the survival of both strains within human macrophages was analyzed. The number of viable intracellular *E. coli* (CE1224.pRM41) bacteria declined rapidly upon internalization. *S. typhimurium* (SL3261.pRM41), entered the macrophages approximately tenfold more efficiently than the *E. coli* strain and multiplied intracellularly within the first 3 h of infection, but was killed effectively upon further incubation (data not shown). It should be noted that these observations are only indicative for the survival of the total population of intracellular bacteria and not for individual bacteria. It has been observed that a significant amount of *S. typhimurium*-containing phagosomes do fuse with lysosomes [34]. The higher numbers of *S. typhimurium* internalized, and the amount of *S. typhimurium* containing phagolysosomes formed, is possibly sufficient to generate the limited amount of MHC/peptide-complexes required for T cell activation, resulting in similar antigen processing kinetics as for *E. coli*. When heat-killed bacteria were used, maximal T cell activation was already observed between 1–3 h after incubation. Probably, the PhoE-TH4 protein within the heat-killed bacterial preparations is directly

Figure 4. Inhibition of the presentation of the T_h epitope via HLA class II by human macrophages, infected with recombinant *E. coli* CE1224 (A) or *S. typhimurium* SL3261 (B) bacteria, by brefeldin A, cycloheximide or cytochalasin D. Macrophages were incubated with peptide 453–464 (1 μM), with CE1224.pRM41 or SL3261.pRM41 bacteria expressing PhoE-TH4, or with CE1224.pRM40 expressing PhoE-CTL4. Viable or heat-killed bacteria (HK) were added at an MOI of 10. After incubation, the macrophages were washed extensively and incubated for 3 h in medium containing gentamycin. The drugs were added 30 min before incubation with the antigens and maintained in the culture medium until fixation. The cells were fixed with glutaraldehyde and the proliferation of the T cell clone JP-F94 was measured. The results are expressed as mean [3H] thymidine incorporation ± SD of triplicate cultures.

accessible for antigen processing after internalization, whereas internalized viable bacteria first have to be degraded following phagosome/lysosome fusion. The time needed for antigen presentation by human macrophages incubated with either viable or heat-killed recombinant *S. typhimurium* SL3261 was comparable to that reported for human monocytes [21]. In contrast, antigen presentation by activated mouse peritoneal macrophages infected with recombinant *E. coli* has been shown to occur within 30–60 min after infection [35]. The non-activated human monocyte-derived macrophages may be less efficient in processing than mature activated mouse peritoneal macrophages. Alternatively, the chimeric PhoE proteins may be more resistant to processing than the chimeric proteins used in the mouse study, which are based on the cytoplasmic carrier protein Crl [35].

Macrophages fixed with glutaraldehyde before the addition of either viable or heat-killed bacteria expressing PhoE-TH4, were not recognized by the CD4+ specific T cell clone. This result indicates that for both viable and heat-killed bacteria, antigen processing occurred exclusively intracellularly after internalization. The observation that cytochalasin D inhibited the presentation of the T_h epitope by macrophages incubated with viable recombinant bacteria underlines the notion that processing of intact bacteria is dependent on phagocytosis [20, 35]. In contrast, antigen presentation by macrophages incubated with heat-killed bacteria was not abolished by cytochalasin D, indicating that PhoE-TH4 within these bacterial preparations is most likely present in small membrane fragments that can be internalized via a cytochalasin D-resistant pathway, *e.g.* pinocytosis. The observation that cycloheximide and brefeldin A inhibit antigen presentation by macrophages incubated with either viable or heat-killed recombinant bacteria suggests the requirement for ongoing synthesis of HLA class II molecules and their subsequent transport from the endoplasmic reticulum to the Golgi apparatus. These findings are consistent with several reports showing that both drugs block class II mediated presentation of whole antigens but not of synthetic peptides [21, 36, 37].

No evidence was found for recognition of human macrophages, infected with recombinant bacteria expressing PhoE-CTL4, by the specific CD8+ T cell clone. This inability of bacteria-infected macrophages to confer presentation of antigenic determinants via class I is in contrast to other reports [19, 20]. In those studies, activated murine peritoneal macrophages were used as antigen-presenting cells, and a class I-restricted T cell epitope was expressed as chimeric protein in *E. coli* or *S. typhimurium* [19, 20]. In addition to the soluble bacterial proteins Crl and MalE, the outer membrane protein LamB was used as carrier protein [20]. Since LamB and PhoE are structurally similar [38], and the amounts of chimeric PhoE and LamB proteins expressed at the bacterial cell surface were both very high, these factors do probably not account for the discrepancy observed. One difference between the studies, which might be of importance is the antigen-presenting cell used. It has recently been shown that murine macrophages can process and present exogenous antigens, either in soluble form or bound to beads, via class I molecules [39]. Since in that study [39], and in those described by others [19, 20], *in vivo* matured macrophages were used as antigen-presenting cell, we hypothesize that the human macrophages used in the

present study, which had differentiated *in vitro* from monocyte to macrophage, have not acquired the potential to process and/or present the CTL epitope upon infection with the recombinant bacteria. The inability of chinese hamster ovary (CHO) cells to present a CTL epitope upon infection with recombinant *S. typhimurium* [7] underlines the proposed cell type specificity. Alternatively, the peptide corresponding to the CTL epitope might not be generated because of negative effects of flanking residues of the carrier protein [25, 40]. Future experiments using *in vivo* matured human macrophages, and insertion of the CTL epitope including its native flanking residues into PhoE, will be directed to discriminate between these possibilities.

5 References

1 Hoiseth, S. K. and Stocker, B. A., *Nature* 1981. *291:* 238.

2 Clements, J. D., Lyon, F. L., Lowe, K. L., Farrand, A. L. and El-Morshidy, S., *Infect. Immun.* 1986. *53:* 685.

3 Poirier, T. P., Kehoe, M. A. and Beachey, E. H., *J. Exp. Med.* 1988. *168:* 25.

4 Newton, S. M. C., Jacob, C. O. and Stocker, B. A. D., *Science* 1989. *244:* 70.

5 Charbit, A., Sobczak, E., Michel, M.-L., Molla, A., Toillais, P. and Hofnung, M., *J. Immunol.* 1987. *139:* 1658.

6 Leclerc, C., Charbit, A., Molla, A. and Hofnung, M., *Vaccine* 1990. *8:* 269.

7 Gao, X.-M., Tite, J. P., Lipscombe, M., Rowland-Jones, S., Ferguson, D. J. P. and McMichael, A. J., *Infect. Immun.* 1992. *60:* 3780.

8 Aggarwal, A., Kumar, S., Jaffe, R., Hone, D., Gross, M. and Sadoff, J., *J. Exp. Med.* 1990. *172:* 1083.

9 Kaufmann, S. H. E., *Annu. Rev. Immunol.* 1993. *11:* 129.

10 Brunt, L. M., Portnoy, D. A. and Unanue, E. R., *J. Immunol.* 1990. *145:* 3540.

11 Kaufmann, S. H. E., Hug, E. and De Libero, G., *J. Exp. Med.* 1986. *164:* 363.

12 Portnoy, D. A., Jacks, P. S. and Hinrichs, D. J., *J. Exp. Med.* 1988. *167:* 1459.

13 Szalay, G., Hess, J. and Kaufmann, S. H. E., *Eur. J. Immunol.* 1994. *24:* 1471.

14 Chiplunkar, S., De Libero, G. and Kaufmann, S. H. E., *Infect. Immun.* 1986. *54:* 793.

15 Stover, C. K., de la Cruz, V. F., Fuerst, T. R., Burlein, J. E., Benson, L. A., Bennett, L. T., Bansal, G. P., Young, J. F., Lee, M. H., Hatfull, G. F., Snapper, S. B., Barletta, R. G., Jacobs, R. W. Jr. and Bloom, B. R., *Nature* 1991. *351:* 456.

16 Pope, M., Kotlarski, I. and Doherty, K., *Immunology* 1994. *81:* 177.

17 Hermann, E., Yu, D. T. Y., Meyer zum Büshenfelde, K.-H. and Fleischer, B., *Lancet* 1993. *342:* 646.

18 Turner, S. J., Carbone, F. R. and Strugnell, R. A., *Infect. Immun.* 1993. *61:* 5374.

19 Pfeifer, J. D., Wick, M. J., Roberts, R. L., Findlay, K. A., Normark, S. J. and Harding, C. V., *Nature* 1993. *361:* 359.

20 Wick, M. J., Pfeifer, J. D., Findlay, K. A., Harding, C. V. and Normark, S. J., *Infect. Immun.* 1993. *61:* 4848.

21 Brett, S. J., Rhodes, J., Liew, F. Y. and Tite, J. P., *J. Immunol.* 1993. *150:* 2869.

22 Janssen, R. and Tommassen, J., *Int. Rev. Immunol.* 1994. *11:* 113.

23 Cowan, S. W., Schirmer, T., Rummel, G., Steiert, M., Ghosh, R., Pauptit, R. A., Jansonius, J. N. and Rosenbusch, J. P., *Nature* 1992. *358:* 727.

24 Hogervorst, E. J. M., Agterberg, M., Wagenaar, J. P. A., Adriaanse, H., Boog, C. J. P., Van der Zee, R., Van Embden, J. D. A., Van Eden, W. and Tommassen, J., *Eur. J. Immunol.* 1990. *20:* 2763.

25 Janssen, R., Wauben, M., Van der Zee, R., de Gast, M. and Tommassen, J., *Int. Immunol.* 1994. *6:* 1187.

26 Tommassen, J., van Tol, H. and Lugtenberg, B., *EMBO J.* 1983. *2:* 1275.

27 Van Binnendijk, R. S., Van Baalen, C. A., Poelen, M. C. M., de Vries, P., Boes, J., Cerundolo, V., Osterhaus, A. D. M. E. and UytdeHaag, F. G. C. M., *J. Exp. Med.* 1992. *176:* 119.

28 Agterberg, M., Benz, R. and Tommassen, J., *Eur. J. Biochem.* 1987. *169:* 65.

29 Scholten, M., Janssen, R., Bogaerts, C., van Strien, J. and Tommassen, J., *Mol. Microbiol. in press.*

30 Heijnen, C. J., Uytdehaag, F. G. C. M., Gmelig-Meyling, F. H. J. and Ballieux, R. E., *Cell. Immunol.* 1979. *43:* 282.

31 Te Velde, A. A., Klomp, J. P. G., Yard, B. A., de Vries, J. E. and Figdor, C. G., *J. Immunol.* 1988. *140:* 1548.

32 Vaudaux, P. and Waldvogel, W. A., *Antimicrob. Chemotherap.* 1979. *16:* 743.

33 Finlay, B. B. and Falkow, S., *Biochimie* 1988. *70:* 1089.

34 Buchmeier, N. A. and Heffron, F., *Infect. Immun.* 1991. *59:* 2232.

35 Pfeifer, J. D., Wick, M. J., Russell, D. G., Normark, S. J. and Harding, C. V., *J. Immunol.* 1992. *149:* 2576.

36 St. Pierre, Y. and Watts, T. H., *J. Immunol.* 1990. *145:* 812.

37 Adorini, L., Ullrich, S. L., Appella, E. and Fuchs, S., *Nature* 1990. *346:* 63.

38 Charbit, A., Ronco, J., Michel, V., Werts, C. and Hofnung, M., *J. Bacteriol.* 1991. *173:* 262.

39 Kovacsovics-Bankowski, M., Clark, K., Benacerraf, B. and Rock, K. L., *Proc. Natl. Acad. Sci. USA* 1993. *90:* 4942.

40 Del Val, M., Schlicht, H. J., Ruppert, T., Reddenhase, M. J. and Kozinowski, U. H., *Cell* 1991. *66:* 1145.