Chemotactic cytokines and tuberculosis
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Introduction
Chemotactic cytokines or chemokines comprise two families of peptide mediators which are involved in the recruitment of leucocytes to areas of inflammation or infection. The prototypic member of the α subfamily which is characterized by a Cys-Xaa-Cys sequence motif, is interleukin (IL)-8. Although principally investigated as a neutrophil chemoattractant, IL-8 is a potent chemoattractant to T lymphocytes both in vitro and in vivo [1]. The β chemokine subfamily is characterized by a Cys-Cys motif and is a more homogeneous group of mediators as many members of this group have actions upon monocytes. The principal β chemokine is monocyte chemotactic protein-1 (MCP-1), which is both chemotactic for monocytes and activates their respiratory burst and increases cytosolic free calcium [2]. Human monocytes express the gene for and secrete both MCP-1 [3] and IL-8 [4].

Understanding the immune response to tuberculosis is increasingly important because this infection causes approximately 3 million deaths worldwide each year [5] and new therapeutic strategies are required in the face of increasing drug resistance. The principal human immune response to Mycobacterium tuberculosis infection is the development of granulomas. These consist of cells of the monocyte lineage including epithelioid cells and multinucleate giant cells together with antigen-specific T lymphocytes. Cytokines such as tumour necrosis factor (TNF) and direct cell-cell interactions are undoubtedly important in the formation of these complex structures [6]. However, before formation of granulomas, mycobacteria are phagocytosed by monocytes and macrophages and then leucocytes are recruited to the area of infection. It seemed probable that the process of phagocytosis might be pivotal in initiating and controlling the subsequent influx of monocytes and T cells. Hence, an in vitro model of M. tuberculosis infection was developed to investigate the transcriptional control of the chemokines following phagocytosis of this pathogen.

MCP-1
The experimental protocol developed and previously published [7] is outlined in Figure 1. A critical feature is performing tissue culture in perfluoralkoxy Teflon vials to prevent adherence to tissue culture plastic and activation of chemokine secretion [8]. In addition to virulent and avirulent strains of M. tuberculosis (H37-Rv and H37-Ra, respectively), studies were performed on Escherichia coli-derived lipopolysaccharide (LPS), a positive soluble control, and yeast-derived zymosan, a positive particulate control. Inert latex beads which do not activate oxidative burst mechanisms or stimulate gene expression or secretion of the proinflammatory cytokines TNF and IL-6, were the negative particulate control.

Abbreviations used: hsp, heat-shock protein; IL, interleukin; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein-1; TNF, tumour necrosis factor.
Phagocytosis resulted in increased gene expression of MCP-1 within 4 h in THP-1 cells, a human monocytic cell line, as detected by northern analysis (Figure 2). MCP-1 mRNA accumulated over a 24 h period and gene expression persisted for at least 5 days. The interesting observation was that such gene expression was independent of the nature of phagocyted particle and could even be induced by inert Latex beads. In addition, the kinetics and magnitude of MCP-1 gene expression were similar in experiments involving the phenotypically more differentiated phagocytic monocyte cell line Mono Mac 6. Studies with actinomycin D and cyclohexamide, a protein synthesis inhibitor, revealed that MCP-1 gene expression was transcriptionally upregulated by the process of phagocytosis [9]. The ability of inert particles as well as pathogens to stimulate the MCP-1 gene is consistent with the observation that monocyte-derived granulomas may form in response to foreign bodies. In mycobacterial granulomas, however, the involvement of T lymphocytes is critical and hence the pattern of IL-8 gene expression and secretion was of particular interest.

**IL-8**

Phagocytosis of *M. tuberculosis* by THP-1 cells resulted after 24 h in 8–15-fold greater secretion of IL-8 than did phagocytosis of zymosan or stimulation by LPS, previously considered to be the most potent stimulus for IL-8 secretion. Such increased secretion was in part attributable to a threefold increase in IL-8 gene expression over the 24 h period. Elevated IL-8 concentrations in tissue culture medium were persistent over 5 days. A similar pattern of IL-8 secretion occurred in Mono Mac 6 cells with altered kinetics. The increase in IL-8 gene expression occurred within 4–8 h. The effect of phagocytosis of *M. tuberculosis* on IL-8 secretion was independent of autologous TNF secretion, which may influence IL-8 concentrations in other systems [10].

The ability of phagocytosis of *M. tuberculosis* to stimulate IL-8 secretion did not appear to be a determinant of mycobacterial virulence. No differences in secreted IL-8 concentrations were found between H37-Rv or H37-Ra following their phagocytosis. Furthermore, prior infection of THP-1 cells by human immunodeficiency virus, a clinically important predisposing factor to tuberculosis infection, did not affect IL-8 gene expression or secretion. But massive IL-8 secretion by monocytes was a specific response to *M. tuberculosis* and did not occur after phagocytosis of *Toxoplasma gondii* which, like latex particles, neither stimulated IL-8 gene expression nor secretion of this cytokine [11].

Thus it appears that the ability of monocytic cells to release this T-cell chemotactic agent following phagocytosis is dependent upon the nature of the phagocytosed particle, and a differential effect may be important in the development of an antigen-specific T cell-mediated immune response. Subsequent investigation concentrated upon the detailed investigation of a specific antigen, the mycobacterial 65 kDa heat-shock protein (hsp), hsp65.

**Mycobacterial hsp65**

Hsp65 is expressed at high levels of mycobacteria within monocytes following phagocytosis. It is thought to provide protection against host oxidative killing mechanisms, as does the analogous protein in *Salmonella typhimurium* [12]. The monocyte can however process mycobacterial hsp65 and presents epitopes on the cell surface to T αβ and γδ lymphocytes via the major histocompatibility complex [13–15]. The question was whether or not mycobacterial hsp65 stimulated monocytes to release the T-cell chemokine IL-8 in addition to processing epitopes via antigen presentation pathways. Recombinant mycobacterial hsp65 (gift of Dr J. Colston, NIMR, London, U.K.) was passed three times over a lipid A column at 4°C to remove LPS [16]. This was checked by *Limulus* amoebocyte assay. Protein integrity was confirmed by SDS/PAGE.
PAGE before quantification by spectrophotometric methods. Studies then demonstrated a heat-sensitive (i.e. protein- nor LPS-mediated) secretion of IL-8 by THP-1 cells exposed to hsp65, answering the question above in the affirmative [17].

Conclusions
Chemokine secretion by monocytic cells following phagocytosis is under tight transcriptional control. Upregulation of MCP-1 gene expression is a non-specific response to phagocytosis, as may be formation of foreign body granulomas. In contrast, gene expression and secretion of the T-cell chemoattractant IL-8 is dependent upon the nature of the organism phagocytosed and occurs at high levels after phagocytosis of M. tuberculosis. Such antigen-specific responses may partly be a consequence of cellular activation by mycobacterial hsp65, which has now been shown to stimulate chemokine secretory and antigen presentation pathways in monocytic cells. In view of the many similar hsps expressed by other pathogens, effects on chemokines may be a common, important host-defence mechanism. Little is known however, about kinetics of intracellular exposure to foreign hsps, which may be critical in development of such immune responses.

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