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EDITORIAL REVIEW

Striking the right balance; the role of cytokines in mycobacterial disease

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Mycobacteria are intracellular parasites that have been the scourge of man since the earliest days of recorded history. They infect monocytic cells and, in susceptible individuals, cause chronic inflammation and progressive scarring [1]. It is clear that individual susceptibility to mycobacterial disease varies greatly, and that some are relatively resistant. Such individuals eliminate mycobacteria through a combination of innate and acquired immunity, which involves bidirectional interactions between macrophages and lymphocytes. However, protection is not only dependent on the host, but also on the aggressiveness of the mycobacterial strain involved.

Macrophages are the first line of defence against mycobacteria, and when infected rapidly become activated [2] and synthesize proinflammatory cytokines, including tumour necrosis factor (TNF), IL-1α and β, IL-6 [3-5] and granulocyte-monocyte colony-stimulating factor (GM-CSF) [6]. It is certain that other cytokines are secreted as well, and so one must be careful before assigning a definitive role to a particular cytokine. Nevertheless, sustained synthesis of TNF, IL-2 and GM-CSF [6-10] is associated with killing, whereas IL-1α and IL-6 can promote mycobacterial growth, at least of *Myco. avium* [7,10]. Various mycobacterial products can activate macrophages. Peptidoglycan and their fragments stimulate IL-1, IL-6 and TNF production (reviewed in [11]). Lipoarabinomannan (LAM) has the same effect [12,13], particularly in the presence of interferon-gamma (IFN-γ) [12]. It is also apparent that LAM has differential effects on macrophages from different sources [14].

Three lines of evidence emphasize the importance of TNF (and possibly other macrophage-derived cytokines) in controlling mycobacterial infections. First, virulent strains of mycobacteria induce less TNF synthesis than less virulent ones of the same species [5,15]; second, LAM from different strains of *Myco. tuberculosis* induce strikingly different levels of TNF and other cytokines [13]; and finally, administration of anti-TNF antibodies to mice infected with bacille Calmette-Guérin (BCG) inhibits hepatic granuloma formation and results in their death from overwhelming infection [16]. Coincidently, this treatment also inhibits hepatic TNF synthesis. These observations demonstrate the importance of macrophage activation in the defence against mycobacteria. This raises questions about the role of IFN-γ, a powerful macrophage activator which could provide a link between innate and acquired T cell-mediated immunity, which was shown many years ago to be important in defence against mycobacteria [17].

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There is evidence for the involvement of IFN-γ at an early stage in the defence, especially from *in vivo* studies on mice which have defined a genetic locus on chromosome 1 (variously called Bcg, Ity, and Lsh [18,19]) which contains a gene (or genes) that determines susceptibility to mycobacteria as well as other intracellular parasites (reviewed in [20]). Macrophages from resistant strains infected with an appropriate intracellular pathogen, secrete factors that induce natural killer (NK) cells to produce IFN-γ, whereas macrophages from susceptible strains lack this capacity [21]. The nature of the factors involved is not certain, but TNF enhances the effect [22]. However, these experimental data should not be over-interpreted, because immune protection against BCG could be qualitatively different from that to *Myco. tuberculosis*, as indicated by experiments with 'CD8 knockout mice' that were shown to be resistant to BCG and susceptible to *Myco. tuberculosis* [23].

Infected macrophages also present antigen to T helper cells. Differences in CD4 T cell epitopic profiles are beginning to emerge between normal purified protein derivative (PPD)-positive individuals and patients with tuberculosis [24], and might be expected to influence the pattern of cytokine secretion and so influence resistance to the infection. This issue has acquired special significance since the identification of CD4$^+$ T cell subsets with a propensity to secrete distinct cytokine profiles: IFN-γ, IL-2, IL-12 in the case of Th1 cells, and IL-4, IL-5 and IL-10 in the case of Th2 cells [25]. Typically, human T cell clones responding to PPD raised *in vitro* correspond to the Th1 phenotype and produce IL-2 and IFN-γ [26], but prior exposure to IL-4 before cloning induces Th0 and Th2 rather than Th1 cells [27]. This suggests that the situation is likely to be more complicated *in vivo*, and this is supported by studies in humans. These have shown that T cells from tuberculosis patients synthesize IL-4 and IL-5 as well as INF-γ and IL-2, when stimulated *in vitro* [28], thus indicating the presence of Th1 and Th2 cells, or of lymphocytes with a much broader cytokine repertoire, resembling the Th0 cell of the mouse [29]. Studies of T cells in the skin of subjects undergoing mycobacterial reactions also suggest the presence of cells capable of producing cytokines associated with both Th1 and Th2 phenotypes [30]. The situation is further complicated by recent observations showing that CD8$^+$ T cells can also be separated into subtypes according to their capacity to secrete different cytokines [31].

Much more needs to be known about defences against mycobacteria, but the observations so far discussed demonstrate an important role for TNF and IFN-γ. However, in this context as in others these cytokines can also contribute to pathogenesis, as exemplified by two papers in this issue of *Clinical and Experimental Immunology*.

Cadranel *et al.* (page 51) report that peripheral blood monocytes from patients express greater numbers of TNF receptors than controls, and that a greater proportion of them are complexed with TNF. This provides evidence for a generalized macrophage activation in TB, and of increased TNF synthesis. These findings complement those of Ogawa *et al.* [32], who had previously shown that patients' peripheral blood monocytes produce more TNF. They also invite comparison with the studies of TNF levels in mice infected with BCG [16].

Wangoo and colleagues (page 43) concentrate on the role of IFN-γ, and demonstrate that it stimulates alveolar macrophages to produce platelet-derived growth factor B chain (PDGF-B), but not transforming growth factor (TGF-β). They also report that supernatants from PPD-stimulated lymphocytes have the same effect, and that this can be blocked by antibodies to IFN-γ. PDGF and TGF-β have previously been localized to sites of pulmonary fibrosis [33–37], and so Wangoo *et al.* use their data to infer that PDGF, rather than TGF-β, is responsible for the progressive fibrosis which is so often the feature of tubercular disease. This is an interesting hypothesis, but more experimental information is needed in its support, because (i) the data refer to the effect of IFN-γ on isolated alveolar macrophages and not to cytokines produced by the cells adjacent to the macrophages in the lesion; and (ii) infected macrophages exposed to mycobacterial products might respond by producing a different set of cytokines, including TGF-β.

Increased expression of PDGF in pulmonary fibrosis has been demonstrated, but it is necessary to be cautious when invoking a causal link between the two. Not least because experimental precedents indicate that PDGF and TGF-β can be expressed simultaneously in models of fibrosis, with PDGF having a relatively minor influence on matrix synthesis, as for example in the Thy 1.1 model of experimental nephritis [38,39].

Nevertheless, it seems reasonably clear that TNF-α and IFN-γ have a dual role in the immunity and pathology of mycobacterial disease; and that different forms of T cell immunity could determine the outcome. Still, the key issue remains: which biochemical events lead to the elimination of *Myco. tuberculosis*? Unfortunately, none of the processes described so far suggest a strong candidate for human tuberculosis.

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