Lack of skin test reactivity to common mycobacterial antigens in human immunodeficiency virus infected individuals with high CD4 counts

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Abstract

Background – T cell response to mycobacterial antigens may be directed against those antigens common to all mycobacteria (group i), those restricted to slow (group ii) or fast growers (group iii), or those which are species- or subspecies-specific (group iv). These responses were assessed by skin testing patients infected with the human immunodeficiency virus (HIV) and healthy controls with reagents derived from different strains of mycobacteria.

Methods – Skin test responses to new tuberculins prepared from *Mycobacterium tuberculosis*, *M avium* serotypes 4 and 8, and either *M intracellulare* or *M flavescens* antigens were evaluated prospectively in 51 HIV infected patients and 67 healthy controls.

Results – Assessment of induration at 72 hours showed absence of skin test response to common mycobacterial antigens in all 27 HIV positive patients with CD4 counts of $\geq 400/mm^3$ (range 400–1594, median 540) compared with 27% reactivity in controls; complete anergy was demonstrated in 24 patients with CD4 counts of $< 400/mm^3$. By contrast, no difference in species or subspecies-specific responses was found between healthy controls and HIV positive patients with CD4 counts of $\geq 400/mm^3$.

Conclusions – Subsets of CD4 + T helper cells are instrumental in determining the balance between cell-mediated and humoral immunity. One T helper subset (TH1) produces cytokines that increase cellular immunity and is stimulated by group i common mycobacterial antigens. Lack of this response, but preservation of responses to species-specific antigens while CD4 counts are near normal, may indicate an early failing of TH1 immunity and explain the increased susceptibility of HIV positive patients to mycobacterial infection early on in the evolution of their HIV infection.

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The two major mycobacterial infections that complicate the progress of patients seropositive for the human immunodeficiency virus (HIV) are *Mycobacterium tuberculosis* and *M avium*. Infections with many mycobacterial species have been identified in association with HIV but only *M tuberculosis* and *M avium* are seen frequently. HIV-associated tuberculosis can occur at any stage of the disease, the clinical presentation depending on the CD4 count and whether infection is primary or due to reactivation. By contrast, *M avium* is only seen in the later stages of HIV infection when the CD4 count is low ($< 100/mm^3$) and then it presents as a disseminated disease. Whether *M avium* disease is due to reactivation of previously acquired infection or to new infection is unclear.

In favour of reactivation is the highly significant association of certain *M avium* serotypes (1, 4, and 8) with disease in HIV;[1] these strains appear distinct from those causing disease in most patients uninfected with HIV and isolates from the environment. In addition, both T cell and B cell responses have been described in non-HIV infected healthy persons against *M avium* infection indicating past exposure and skin sensitisation.[2 3] By contrast, the finding of a higher rate of *M avium* in young persons infected with HIV,[4] documented prior respiratory or gastrointestinal tract colonisation in one third,[5] multiple serotypes in 10%,[1] and a geographical association between environmental and HIV associated prevalences of these organisms argue in favour of newly acquired infection.[6] The recent implication of potable hospital water as a source of *M avium* infection in seven patients with *M avium* complex infection using pulsed gel field electrophoresis typing provides further support for this theory.[7]

Mycobacterial antigens may be grouped according to immunodiffusion analysis[8] (table 1). Group i antigens are common to all mycobacteria whereas group iv antigens are species or subspecies-specific. In immunocompetent subjects cell mediated responses to common mycobacterial antigens are associated with protection from tuberculosis; such responses are lost when clinical tuberculosis develops. With advanced tuberculous disease, patients may also lose their skin test responses to species-specific antigens present in tuberculin. Purified protein derivative (PPD) contains mainly group i and ii antigens and is not species-specific. In order to investigate the state of immunity in HIV seropositive individuals to mycobacterial infection we have carried out skin testing with four new tuberculins[9] and

Table 1   Classification of mycobacterial antigens according to immunodiffusion analysis

		Groups of antigens			
		i	*ii*	*iii*	*iv*
Slow growers	M tuberculosis M avium 4 M avium 8 M intracellulare				M tuberculosis M avium 4 }a }b M avium 8 M intracellulare
Fast growers	M flavescens M chelonei				M flavescens M chelonei
	M vaccae M leprae				M vaccae M leprae

a = some cross reactivity exists; b = small amount of cross reactivity exists; group i = common mycobacterial antigens present in every mycobacterial species; group ii = slow grower associated antigens present in all slow growing species; group iii = fast grower associated antigens present in most fast growing species; group iv = species-specific antigens showing some sharing between very closely related species.

have compared the results with those achieved in an HIV seronegative control group.

Methods
The new tuberculin skin test reagents were prepared as previously described.[9] Briefly, suspensions of live *M tuberculosis* (T), *M avium* serotype 4 (A-4) and serotype 8 (A-8), *M intracellulare* (AC), and *M flavescens* (F) were cultured on non-antigenic medium, sonicated in borate-buffered saline containing Tween 80, and sterilised by filtration. Protein concentrations for skin test use were standardised to 2 μg/ml.

Details of age, ethnic group, country of birth, BCG status (and presence of scar), medications and history of any contact with, or illness due to, tuberculosis were collected from healthy volunteers and from HIV positive patients (together with the CD4 count measured within the preceding two months). A history of skin ulceration to tuberculin testing was an exclusion criterion; in addition, healthy volunteers were excluded if they had a previous history of tuberculosis or disease due to other mycobacteria or were on immunosuppressive therapy. A total of 51 HIV positive patients (aged 18–61, median 33 years, 50 men) were enrolled. Of these, 24 had CD4 counts of < 400 cells/mm³ (4–375, median 133 cells/mm³) and 27 had CD4 counts of ≥ 400/mm³ (400–1594, median 540/mm³). In addition, 46 healthy HIV negative controls (aged 20–28, median 21 years, 22 men) were skin tested. 0.1 ml of each of four reagents (T, A-4, A-8, and AC) was injected intradermally into the volar aspects of the forearms. In a further 21 healthy volunteers (aged 20–55, median 22 years, all men) skin testing was performed with F (derived from the fast grower, *M flavescens*) substituted for AC to confirm immune recognition of common mycobacterial antigens (table 1).[10] All subjects tested were born in the

British Isles and there was no difference between the groups according to BCG status. The size of intradermal induration (mm) was measured at 72 hours and a positive result was taken to be ≥ 2 mm. Differences in response between groups were assessed by the two tailed Fisher's exact test.

Results
The results are shown in table 2. In the healthy controls there were no significant differences between numbers responding to all four reagents or to 1–3 reagents when *M intracellulare* reagent AC was substituted for *M flavescens* reagent F, suggesting that response to all four reagents was due to immune recognition of common mycobacterial antigens rather than to (group ii) antigens common to slow growing mycobacteria. None of the 51 HIV positive patients responded to all four skin test reagents, whereas 18 (27%) of the 67 healthy controls did so (p < 0.0001). Of these HIV positive individuals none of the 24 patients with CD4 counts of < 400 cells/mm³ responded to any of the reagents, differing significantly from those with more CD4 cells (p = 0.0002) and from healthy controls. In HIV positive patients with CD4 counts of ≥ 400 cells/mm³ 11 (41%) of 27 responded to at least one reagent, and in this respect they did not differ significantly from the healthy controls (35 of 67 (52%), p = 0.12). Skin test anergy was more frequently encountered in HIV positive patients with CD4 counts of ≥ 400 cells/mm³ than in healthy controls (59% versus 20%, p < 0.001), but the most striking finding was the complete absence of response to all four antigens (p < 0.005) in these patients, suggesting a total lack of recognition of common mycobacterial antigens.

Different skin test responses were observed between antigens derived from serotypes 4 and 8 of *M avium*. In the control group there were 22 (33%) responders to A-4 amongst those reacting to 1–3 reagents, and in the HIV positive group with CD4 counts of ≥ 400 cells/mm³ there were seven responders (26%). Responders to A-8 were 10 of 67 (15%) and four of 27 (15%), respectively, in the two groups.

Discussion
Most individuals who respond to all four skin test reagents in a set such as that used in this study are reacting to antigens common to all mycobacterial species (group i in table 1), which can be confirmed by further testing such individuals with reagents prepared from rare species of mycobacteria.[10] Although the antigenic epitopes recognised by cellular immunity

Table 2   Reaction of HIV positive patients and healthy controls to mycobacterial antigens

	Controls (n = 46)	HIV positive patients		Healthy volunteers (substituting M flavescens for M intracellulare (n = 21))
		CD4 ≥ 400 (n = 27)	CD4 <400 (n = 24)	
All 4 antigens	12 (26%)	0 (p <0.005)	0 (p = 0.002)	6 (29%)
No antigens	9 (20%)	16 (59%) (p <0.005)	24 (100%) (p <0.0001)	5 (24%)
1–3 antigens	25 (54%)	11 (41%) (p = 0.1)	0 (p <0.0001)	10 (48%)

p values compared with healthy controls (Fisher's exact test).

differ from those binding to antibodies in most cases, numerous studies of skin testing and lymphocyte transformation have shown them to conform to the groups shown in table 1.[10-12] No reaction to any of the reagents indicates no previous exposure (very unlikely in adults), homeostatic regulation by common mycobacterial antigens of responses to low dose antigen challenge associated with HLA-DR3 status, or impaired T cell immunity. Response to some, but not all, reagents indicates recognition of individual species by their specific antigens,[11] or may be due to responsiveness to slow grower associated antigens (table 1), as shown in a Kuwaiti population.[10] All four reagents used in the major part of our study were prepared from slow growing species containing group ii antigens as shown in table 1. Individuals who respond to all of them may have done so through cellular recognition of either common mycobacterial or slow grower associated antigens, and the additional control group tested with F, prepared from the fast grower *M flavecesens*, which lacks group ii antigens, were included to resolve this.

Our results show complete absence of skin test responses to common mycobacterial antigens in HIV seropositive patients unrelated to their CD4 cell counts. T cell recognition of these antigens is associated with protective immunity, probably represents a TH1 mediated response,[13] and the lack of such responses in our patients may partly explain the increased susceptibility to tuberculosis even at early stages of HIV disease (CD4 count $\geq 400/$ mm^3). Responsiveness to other mycobacterial antigens does depend on T cell count since there is a significant difference in responses to them between patients with more than or less than 400 CD4 cells/mm^3.

Although it is customary to assume that immune changes found in HIV seropositive individuals are the result of immunodeficiency induced by the virus, the possibility has to be considered that they may be a marker of HIV susceptibility as suggested by recent serological findings.[14] Hence, lack of cellular responsiveness to common mycobacterial antigens may reflect the pivotal role of TH1 cells in protection from HIV infection. A body of circumstantial evidence is accumulating that cellular responses to these mycobacterial antigens,[15] which include the major stress proteins, are mediated through TH1 rather than TH2 cells. Recognition of common mycobacterial antigens may therefore be a simple marker of protection from HIV disease, a viewpoint in agreement with that of Clerici and Shearer relating to T cell function and disease susceptibility.[16]

Serotyping is dependent on chemically distinct surface glycolipids. The fine specificity of skin test responsiveness to the different serotypes of *M avium* is surprising. Although this has been shown before for the subspecies based on immunodiffusion analysis,[17] it has not been previously demonstrated for Schaefer's agglutination serotypes. In a situation analogous to tuberculin sensitivity, responsiveness may indicate prior infection and the presence of latent

M avium infection. With advancing immunosuppression, reactivation of dormant foci may then result in disseminated infection. If this is true, the occurrence of *M avium* disease later than tuberculosis in the progression of HIV might be due to the prolonged survival of cellular responses to *M avium* surface antigens. Hence, a positive *M avium* skin test may be a marker of later reactivation.

There was no significant difference between responders to species-specific antigens in both the HIV seropositive individuals and healthy controls, suggesting that our results are not explained by non-specific loss of cutaneous delayed type hypersensitivity – for example, to mumps and tetanus toxoid antigens – in HIV positive patients with CD4 counts of $\geq 400/$ mm^3.[18] However, when examining patients with HIV infection there is a higher proportion of responders to serotype 4 than to serotype 8, which mirrors the relative prevalence of these serotypes amongst *M avium* strains recovered from patients with HIV infection in the UK.

Responsiveness to common mycobacterial antigens (and hence protective immunity) can be boosted by BCG or killed *M vaccae*. Immunotherapy with killed *M vaccae* has been shown previously to enhance T cell recognition of common mycobacterial antigens in adults with lepromatous leprosy or pulmonary tuberculosis as judged by skin test and lymphocyte transformation tests.[19 20] Our results support the need for similar studies to be conducted in HIV positive patients.[21]

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