

**Answers**

**Problem solving exercise: an immunosuppressed patient**

Q1 What are the molecular mechanisms leading to fever?

A1 The cytokines TNF- $\alpha$  and IL-1, produced at sites of inflammation, circulate in the blood and reach the hypothalamus. In the hypothalamus, TNF- $\alpha$  and IL-1 induce prostaglandin synthesis. Via neural mechanisms, vasoconstriction and shivering are induced, thus decreasing heat dissipation and increasing heat production.

Q2 Comment on any abnormalities in these results.

A2 The only abnormality is a raised C-reactive protein.

Q3 What are the physiological functions of C-reactive protein?

A3 C-reactive protein is an acute phase protein released by the liver in response to cytokines, such as IL-1, IL-6 and TNF- $\alpha$ . C-reactive protein acts as an opsonin and may also activate the complement cascade.

Q4 What is the likely identity of these organisms?

A4 *Mycobacterium tuberculosis*

Q5 What is the name given to this stain?

A5 Ziehl-Neelsen stain.

Q6 Are there any other similarly staining organisms?

A6 *Mycobacterium avium intracellulare* and *Mycobacterium bovis* are two other mycobacterial species which can cause similar infections to tuberculosis. *Mycobacterium avium intracellulare* is seen mainly in the immunosuppressed.

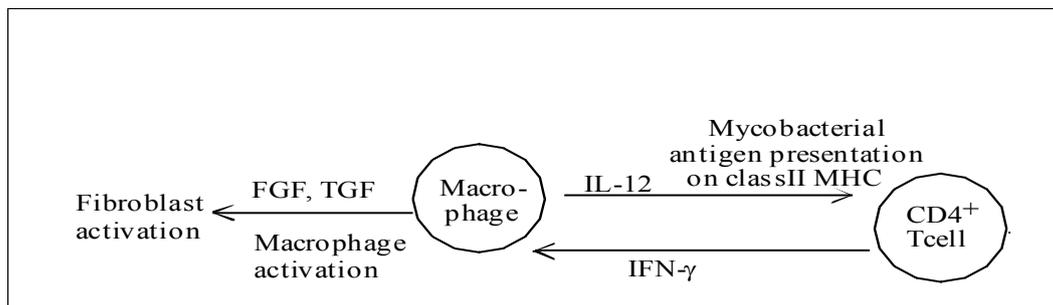
Q7 Describe the appearance shown.

A7 A circumscribed granuloma is present. Within this, occasional multinucleate giant cells (Langhans cells) and numerous epithelioid macrophages are present, with a central area of caseous necrosis. A ring of fibrosis encircles the granuloma.

Outside this, large numbers of small lymphocytes are seen. In this context, these are most likely to be T lymphocytes.

Q8 Explain the immunological processes that are causing this histological appearance.

A8 Once phagocytosed, tuberculosis bacilli are resistant to killing by macrophages and the mycobacteria remain within endosomes of the cell (used for presentation of antigen on class II molecules). Infected macrophages continually present mycobacterial peptides in association with class II MHC to CD4<sup>+</sup> T lymphocytes. The macrophages also produce IL-12, inducing CD4<sup>+</sup> T lymphocyte differentiation into T<sub>h</sub>1 cells. Activated CD4<sup>+</sup> T lymphocytes migrate to the site of mycobacterial infection and produce cytokines, such as interferon- $\gamma$ , which attract and activate macrophages. The macrophages then form a granuloma (a collection of activated macrophages) at the site of infection. In turn the macrophages, if sufficiently activated can kill the intracellular mycobacteria, and release toxic enzymes, reactive oxygen species and other inflammatory mediators, causing tissue necrosis. The macrophages also produce cytokines and growth factors activating surrounding fibroblasts to give the characteristic ring of fibrous tissue seen around granulomas.



Q9 Do you know any risk factors that underlie this disorder?

A9 Risk factors for tuberculosis include: Immunosuppression, old age, origin from Asian subcontinent, homelessness/poor housing.

Q10 Can you correlate these risk factors with any of his previous problems?

A10 His previous 'flu-like illness may have represented seroconversion for HIV. The ensuing loss of CD4<sup>+</sup> T lymphocytes, causing immunosuppression, could have put him at risk of tuberculosis.

Q11 Comment on each of the abnormalities in these results and provide possible explanations for them.

A11 There is a decrease in the number of red blood cells and haemoglobin, i.e., anaemia. This may be related to chronic disease. Cytokine production in chronic disease may suppress bone marrow red blood cell production. Decreased numbers of platelets are seen because HIV can infect a proportion of megakaryocytes. The decreased number of lymphocytes is due mainly to a decrease in CD4<sup>+</sup> T lymphocytes, due to direct infection by HIV, leading to cytolysis by CD8<sup>+</sup> T lymphocytes. A small decrease in the numbers of B lymphocytes and CD8<sup>+</sup> T lymphocytes is likely to represent decreased proliferation of these cell types due to a decrease in "help" from CD4<sup>+</sup> T cells. As a consequence of the large decrease in CD4<sup>+</sup> T lymphocytes, the CD4:CD8 ratio (normally 2:1) is reversed. Numbers of monocytes are normal. Monocyte numbers are often decreased in HIV, as monocytes express CD4. However, the tuberculosis infection may have caused a small increase in the number of monocytes, via the actions of inflammatory cytokines on the bone marrow, bringing it back into the normal range. There is a raised CRP, as it is an acute phase protein (see answer to Q3).

Q12 What further test would now be appropriate?

A12 A specific diagnostic test for HIV is required, such as an ELISA test to detect anti-HIV antibody in the patient's serum.

Q13 What are the principles of an ELISA test? Provide 6 sets of appropriate phrases to explain what is occurring in each step of this test (appropriate for boxes 1 to 6 in [Figure C](#) [please do not write on the photograph]).

A13 For this ELISA, the antigen (gp120 in this case) is adsorbed to the wells. The antibody in question (test serum) is added to the wells (step 1). Following incubation with this antibody, the wells are washed thoroughly (step 2). A secondary antibody (in this case an anti-human antibody) labelled with an enzyme is used to detect any of the primary antibody, which is bound to the antigen in the wells (step 3). Unbound secondary antibody is removed by washing thoroughly (step 4). Substrate for the enzyme is added (step 5) and becomes converted to a coloured substance, detectable by an ELISA plate reader, in wells where the primary antibody has bound to the adsorbed antigen (step 6).

Q14 How do you think he contracted this condition?

A14 It is most likely that HIV was contracted from the blood transfusions in Africa. However, other possibilities include sexual transmission (heterosexual – particularly in Africa – or homosexual) and intravenous drug abuse.

Q15 Explain the interaction between this viral infection and the CD4<sup>+</sup> T cell changes.

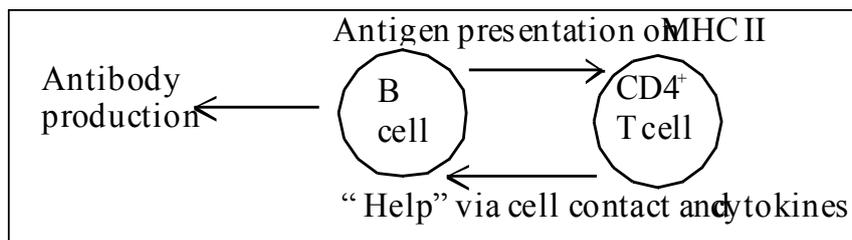
A15 HIV infects CD4<sup>+</sup> T lymphocytes. As shown in **Figure G**, viral gp120 binds to surface CD4 molecules, allowing viral entry into the cell. CD8<sup>+</sup> T lymphocytes detect these infected cells and lyse them in order to remove the virus.

Q16 Explain the changes in CD4<sup>+</sup> T cell count shown on the graph.

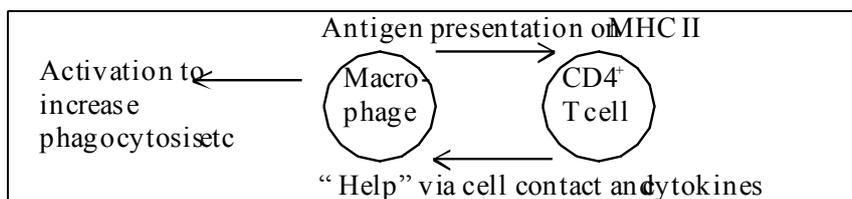
A16 2 - 6 weeks after initial infection, there is a significant decrease in the numbers of CD4<sup>+</sup> T lymphocytes. This occurs as an immune response against HIV is initiated. The CD8<sup>+</sup> T cell response, in particular, is responsible for the killing of large numbers of HIV infected CD4<sup>+</sup> T cells. This initial CD4<sup>+</sup> T cell killing contains the virus for a number of years and infection of further CD4<sup>+</sup> T cells, with subsequent killing by CD8<sup>+</sup> T cells, occurs slowly, accounting for the gradual decrease in the numbers of CD4<sup>+</sup> T cells seen on the graph.

Q17 In what ways are CD4<sup>+</sup> T lymphocytes central to immunological processes, in particular with respect to interactions of CD4<sup>+</sup> T-cells with (i) B cells, (ii) macrophages and (iii) CD8<sup>+</sup> T cells?

A17 (i) B cells cannot become fully activated in response to (T-dependent) antigenic stimuli in the absence of CD4<sup>+</sup> T cell "help".



(ii) Macrophages require CD4<sup>+</sup> T cell "help" in order to become activated.



(iii) CD4<sup>+</sup> T cells produce cytokines. These cytokines help CD8<sup>+</sup> T cells become activated and respond to antigen by lysing infected cells.

Q18 To which organisms may a deficiency in each of these responses give increased susceptibility?

A18 (i) A deficiency in the T<sub>h</sub>2 axis of immunity will give increased susceptibility to extracellular bacteria and some parasites (especially helminths).

(ii) A deficiency in the  $T_{h1}$  axis of immunity will give increased susceptibility to intracellular bacteria and parasites, for example tuberculosis and leishmania.

(iii) A decrease in the CD8 axis of immunity will give increased susceptibility to many viral infections and certain intracellular parasite infections, such as malaria.

Q19 What are the likely causes of death?

A19 The potential causes of death are tuberculous meningitis (seen as granulomas and giant cells in [Figure E](#)) and bacterial bronchopneumonia (seen in [Figure F](#)), both of which are likely to be secondary to HIV-induced immunosuppression.

Q20 Explain why there is an increased susceptibility to bacterial infection, giving the appearances in [Figure F](#).

A20 As discussed in questions 17 and 18, the decrease in  $CD4^+$  T cell numbers, will lead to a decreased B cell response to bacterial antigen.

Q21 What would be the effects of infection with this virus on  $CD4$ -expressing (i) monocytes and (ii) megakaryocytes?

A21 (i) HIV may infect monocytes and macrophages, leading to their destruction by  $CD8^+$  T lymphocytes. A decreased number of monocytes/ macrophages may impair responses against many organisms that require phagocytosis for their destruction and may also contribute to the high incidence of tuberculosis seen in  $HIV^+$  individuals.

(ii) Megakaryocytes are large cells in bone marrow that give rise to platelets. HIV infected megakaryocytes may also be killed by  $CD8^+$  T lymphocytes. The decreased number of megakaryocytes will lead to decreased platelet production, which, in turn, will impair thrombosis, possibly leading to uncontrolled bleeding.