Tuftsin deficiency in AIDS

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Tuftsin is an endogenous tetrapeptide that stimulates phagocytosis and is released from the Fc fragment of IgG by a splenic endocarboxypeptidase. Tuftsin activity and splenic function were measured in 21 patients with AIDS, 7 patients with AIDS-related complex (ARC), 22 patients who had undergone splenectomy, and 37 healthy volunteers. There was a significant inverse correlation between tuftsin activity and splenic function in all subjects. Tuftsin activity was significantly lower in patients with AIDS, ARC, and in those who had undergone splenectomy compared with healthy volunteers. Tuftsin deficiency may contribute to the risk of bacterial infection in symptomatic HIV-positive individuals.

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Introduction

Although the hallmark of AIDS is a depletion of CD4 T lymphocytes, other immunological abnormalities have been reported—eg, decreased monocyte/macrophage chemotaxis, altered cytokine release, reduced natural-killer cell activity, and increased polyclonal B-cell activation.[1] B-cell dysregulation may be a cause of *Streptococcus pneumoniae* pneumonia, which is found more frequently in patients with AIDS.[2] *Strep pneumoniae* infections are associated with splenectomy[3] and with the hyposplenism of sickle-cell anaemia.[4] The protective effect of the spleen may be because of an endocarboxypeptidase that acts with a leukokininase on the outer membrane of phagocytic cells, to release a tetrapeptide (Thr-Lys-Pro-Arg, tuftsin) from the CH2 domain of IgG.[5,6] Serum tuftsin concentrations fall after splenectomy,[7] and are low in sickle-cell anaemia.[8] Tuftsin also stimulates the bactericidal activities of phagocytic cells.[9]

We have measured serum tuftsin activity in patients with AIDS to examine if a deficiency could be another immunological risk factor for infection.

Patients and methods

21 patients with AIDS (15 males, 6 females; mean age 31·4 years, range 24–67), 7 patients with AIDS-related complex (ARC, 5 M, 2 F; 27·4, 23–39), 22 patients who had had a splenectomy (12 M, 10 F; 51·7, 23–77), and 37 healthy volunteers (21 M, 16 F; 28·6, 16–40) took part in the study. AIDS and ARC were diagnosed in accordance with the Centers for Disease Control.[10,11] Of 21 patients with AIDS, 17 had a history of intravenous drug use, 2 were homosexual, and 2 heterosexual. Of 7 patients with ARC, 6 were intravenous drug users, and 1 was homosexual.

HIV seropositivity was confirmed by ELISA ('Enzignost-Anti-HIV-Micro', Behringwerke, Marburg, Germany) and western blots ('New LAV-Blot HIV 1 and 2', Pasteur Diagnostics, Paris, France). In patients with AIDS, CD3, CD4, and CD8 lymphocytes were measured by automated flow cytofluorimetry ('Epics Profile', Coulter Electronics, Hialeah, USA) with FITC-conjugated T3, T4, and T8 monoclonal antibodies (Coulter Immunology, Hialeah, USA), respectively.

Tuftsin activity

10 mg gamma-globulin was isolated from each subject by ammonium sulphate precipitation and dialysis in 0·1 mol/l phosphate buffer (pH 8·1) and then digested at 37°C for 1 hour with 0·5 mg trypsin, in a final volume of 2·5 ml 0·1 mol/l phosphate buffer (pH 8·1) to cleave tuftsin. 4 volumes of 95% ethyl alcohol were added and the alcoholic extract was evaporated under nitrogen. The residue was dissolved in 0·25 ml Krebs-Ringer solution and the precipitate was separated by centrifugation (3000 *g*, 4°C, 30 min).

Tuftsin was assayed by measuring its ability to stimulate phagocytosis of opsonised *Staphylococcus aureus* by neutrophilic granulocytes from healthy volunteers.[12] Controls for tuftsin activity were (a) Krebs-Ringer solution; (b) 0·3 μmol/l synthetic tuftsin-Krebs-Ringer solution (Sigma, St Louis, USA); and (c) Krebs-Ringer solution containing tuftsin extracted from sera from healthy volunteers with known tuftsin activity. All assays were in duplicate. Slide preparations were stained by the May-Grűnwald-Giemsa method. The percentage of granulocytes that contained $\geqslant 1$ *Staphylococcus* was calculated by counting (magnification × 1000) 1000 cells per subject by 2 observers who were unaware of the origin of the samples (interobserver correlation index, 0·96). Tuftsin activity for each sample was calculated by subtracting the percentage of *Staphylococcus*-positive cells in the Krebs-Ringer control.

Splenic function

Splenic function was measured by counting pitted red cells.[13] Venous blood from each subject was mixed with 0·5 ml 3% buffered-glutaraldehyde solution (pH 7·4). 1000 red blood cells were examined in a wet preparation (magnification × 1000) with a direct interference contrast microsocpe (Leitz 'Dialux 20', equipped with Nomarsky optics). The percentage of cells with one or more membrane abnormalities visible as "pits", under interference microscopy, was calculated.

Statistical analysis

Statistical analysis of results was by the two-tailed Wilcoxon rank sum test for unpaired data and the Spearman correlation test. Data are given as mean (SD) unless otherwise stated.

Results

Tuftsin activity in the four groups studied is shown in the figure. Compared with healthy volunteers (21·6% [6·5]),

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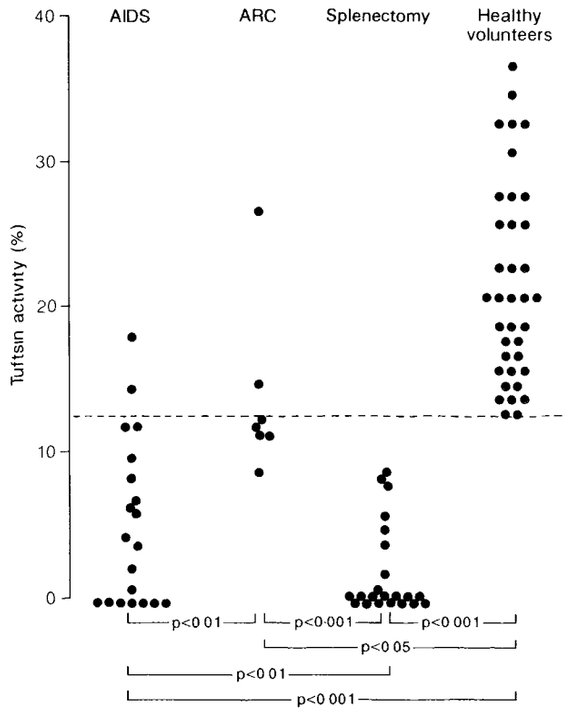

Serum tuftsin activity in study groups.

tuftsin activity was found to be significantly reduced both in patients with AIDS (5·7% [5·6]) and in the splenectomy group (2·0% [3·2]). In patients who had a splenectomy, tuftsin activity was significantly lower than in patients with AIDS. In 2 patients with AIDS, tuftsin activity was above the lower limit of the control range. No difference in clinical features or laboratory data was found between these 2 and the remaining cases. In patients with ARC, tuftsin activity (14·1% [5·9]) was significantly lower than that of healthy volunteers and significantly higher than that of AIDS and splenectomised patients.

Pitted red cell counts in patients with AIDS (6·0% [11·6]) were significantly higher ($p < 0·05$) than in healthy volunteers (0·6% [0·3]) and significantly lower ($p < 0·001$) than in the splenectomy group (28·4% [12·6]). In patients with ARC, pitted red cell counts (1·0% [0·3]) did not differ from healthy volunteers but were significantly lower than patients both with AIDS ($p < 0·05$) and who had had a splenectomy ($p < 0·001$). An inverse correlation between tuftsin activity and percentage of pitted red cells was found for all subjects studied ($z_s = -0·75$; $p < 0·001$).

There was no correlation between either tuftsin activity or percentage of pitted red cells, and absolute numbers of CD3, CD4, or CD8 lymphocytes.

Discussion

Splenomegaly is a common clinical finding in AIDS,[14] but may be associated with functional asplenia.[15,16] A defect in splenic reticuloendothelial function was reported in 11 of 15 individuals with AIDS and it has been suggested that this abnormality, together with other immune defects, may facilitate spread of opportunistic infection.[17]

We have shown reduced tuftsin activity in patients with AIDS and ARC. The inverse correlation between tuftsin activity and pitted red cell counts accords with the suggestion that the spleen may regulate tuftsin activity.

There was no correlation between tuftsin activity and CD4 cell count in patients with AIDS and ARC, possibly because after progression to AIDS, CD4 cell counts tend to reach a plateau.[18] Thus, if CD4 cells are sufficiently reduced in number (all our patients had counts $< 0·4 \times 10^9$/l) there may not be a strict correlation with other clinical or haematological variables.

The clinical counterpart of serum tuftsin deficiency may be the risk of community-acquired bacterial infection, especially with *Strep pneumoniae*. In this study, 6 of 21 patients with AIDS had one or more episodes of pneumococcal infection. Pre-treatment of mice, infected with *Strep pneumoniae* and who had had a splenectomy, with synthetic tuftsin significantly improved survival rate.[19] Tuftsin has been given to human beings without toxic effects and with a significant increase in polymorphonuclear leucocytes and CD4 lymphocytes in peripheral blood.[20] In one patient with AIDS, in whom other therapeutic regimens had failed, tuftsin induced a dramatic clinical improvement that lasted until the substance was given at high doses.[21]

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