Asian Pacific Journal of Allergy and Immunology (1993) 11 : 131-133

Enhanced Phagocyte Chemiluminescence in Asymptomatic HIV Infection

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SUMMARY **Seventeen asymptomatic HIV infected patients were studied for their phagocyte function *in vitro*, in comparison with that of eight normal healthy persons. Chemiluminescence was measured using whole blood by means of a microtitreplate luminometer. Light indices, cumulative light indices and rapidity of responses were recorded. The patients had a lower phagocyte count ($13.17 \pm 0.85 \times 10^9$/l) but a more rapid and intense chemiluminescence response. The latter was demonstrated by a higher peak light index and cumulative response. The observed enhanced phagocyte activity may reflect an early failure of T cell regulatory functions, or a compensatory mechanism in response to the underlying immunodeficiency.**

The human immunodeficiency virus (HIV) commonly affects helper T lymphocytes bearing CD4 markers on their surfaces, causing functional and quantitative depletion. The end result is that of an impairment of cell-mediated immunity, resulting in complications ranging from viral, fungal to mycobacterial infections. Infection with bacterial pathogens, however, occurs not uncommonly, suggesting concurrent phagocyte defects. Studies produced conflicting results-indicating that phagocyte functions could be enhanced[1,2] or depressed[3,4].

The present study was designed to examine phagocyte functions using a chemiluminescence system with minimal disturbance to cells through handling. Asymptomatic HIV infected persons with apparently relatively intact immune systems were compared with normal controls.

MATERIALS AND METHODS

Patients

Seventeen consecutive HIV infected persons followed up at the Special Medical Consultation Clinic of Queen Elizabeth Hospital, Hong Kong, between January and August 1991 were recruited. They were all asymptomatic males who could be grouped in Class II of the 1987 CDC classification format. Freshly drawn whole blood was heparinized and diluted 1:1 in phenol red-free Hanks' Balanced Salt Solution (HBSS) before assay. Similarly blood samples from eight normal healthy male adults were drawn and assayed.

Phagocyte chemiluminescence

Zymosan-induced luminal-enhanced phagocyte chemiluminescence was measured using whole blood in a microtitreplate format as described.[5] Briefly, 200 µl of diluted whole blood was dispensed into each well of a microtitreplate [Dynatech] containing 20 µl opsonized zymosan and 20 µl luminol at 10^{-3} M. All tests were performed in triplicates. After continuous shaking at 37°C for five minutes, the plates were transferred to a microtitreplate luminometer [Amerlite] where readings were recorded every 15 minutes for 6 hours. Light indices of resting cells were subtracted from each pair of triplicated samples.

The following parameters were measured: phagocyte count (taken to be equivalent to the number of neutrophils plus monocytes, using an automated analyser), peak light

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index and cumulative response over 6 hours corrected to 10^6 phagocytes; time lag (difference between test and a standard control in reaching the peak response). Variables were expressed as mean ± standard deviation. The Student's *t* test was used to compare the results, and a p value of < 0.05 was taken to be significant.

RESULTS

The mean age of the study population was 35 (range 24-73) years. All patients contracted HIV by sexual transmission: 11 were homosexuals, and the other 6 were heterosexuals. The CD4 count at the time of study ranged from 186 to 939/mm^3 with a mean value of 428/mm^3. Mean CD4 percentage was 21.9 ± 6.6. Three patients were receiving zidovudine 500-600 mg/day.

The patients generally had a lower phagocyte count than controls (3.17 ± 0.95 *vs* $5.09 \pm 0.99 \times 10^9$/l; $p < 0.05$). Table 1 summarizes the phagocyte responses under the experimental conditions. In general, HIV infected individuals had a lower total phagocyte count. However, a more rapid and stronger chemiluminescence response could be demonstrated both in terms of peak values and cumulative measurement. Fig. 1 illustrates the contrast between one HIV-infected and one normal person. The CD4 count and zidovudine treatment were not related to the observed chemiluminescence responses either positively or negatively.

DISCUSSION

Chemiluminescence measurement is a simple way of assessing phagocyte function *in vitro*, especially in reflecting reaction of the myeloperoxidase-hydrogen peroxide (MPO-H_2O_2) system. Freshly drawn

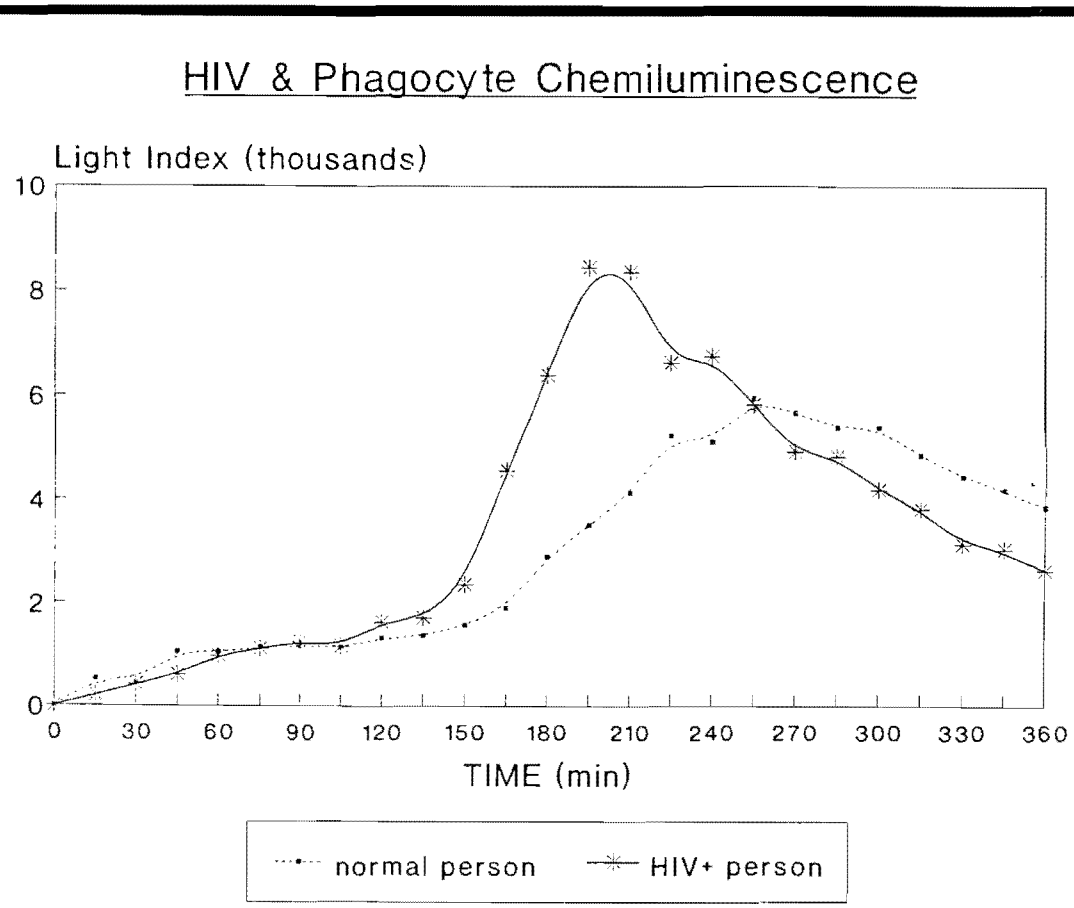

Fig. 1 Phagocyte chemiluminescence responses of an HIV infected persons as compared with a normal healthy individual. Light indices in the figure have been amplified to better illustrate the responses.

Table 1. Zymosan-induced luminol-enhanced phagocyte chemiluminescence of HIV + and normal persons.

	HIV (+) (n = 8)	Normal (n = 17)	P
Total phagocyte count ($\times 10^9$/l)	3.17 ± 0.95	4.09 ± 0.99	< 0.05
Peak light index ($\times 10^{-5}$)	5343 ± 1575	3139 ± 789	< 0.001
Cumulative response (arbitrary units)	59.46 ± 17.05	45.60 ± 8.00	< 0.001
Mean time lag	+24.3 min	-53.8 min	< 0.001

whole blood was studied in order to minimize the element of manipulation. The use of a microtitreplate system had the additional advantage of simplicity and multiple sampling in the same setting.

The present study demonstrated leukopenia and an enhanced phagocytic function in asymptomatic HIV infection measured by whole blood chemiluminescence. Hematologic abnormalities, particularly that of cytopenia[6] have been reported in association with HIV/AIDS. Marrow suppression, effects of drugs, infection and autoimmune destruction[7] through production of anti-neutrophil antibody, are some of the possible underlying mechanisms. Impairment of phagocyte function has been investigated by different workers, mostly in AIDS-symptomatic cases rather than among asymptomatic HIV infected persons.[1,2,8] Such impairment could be explained by myelodysplasia directly resulting from longstanding HIV infection.

The enhanced phagocyte chemiluminescence reported in this study is an interesting phenomenon. D'onofrio *et al.*[3] observed that some AIDS patients had giant neutrophils with increased peroxidase activity. Enhanced candidacidal activity of polymorphonuclear cells has been described as an early defect in HIV infection. Recently, a synthetic peptide derived from part of an HIV antigen sequence was shown to increase chemiluminescence and NBT reduction of normal polymorphs.[9] This evidence supports our current findings. Enhanced phagocyte activation may occur early in HIV disease, reflecting a failure of T cell regulatory functions. Alternatively, this could be a compensatory mechanism whereby phagocyte activation was heightened in the presence of immune deficiency. The significance of the enhanced phagocyte function needs to be established by longitudinal studies and corelated with the clinical outcome.

ACKNOWLEDGEMENTS

The authors are greatly indebted to all nurses of the AIDS Counselling & Health Education Service of the Department of Health for their assistance and care for the patients described in this work.

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