

Neopterin and Alpha and Beta Interleukin-1 Levels in Sera of Patients with Human Immunodeficiency Virus Infection

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Levels of neopterin and alpha and beta interleukin-1 (IL-1) in sera of normal controls, asymptomatic intravenous drug abusers, homosexuals, and patients with lymphadenopathy or acquired immunodeficiency syndrome were measured. Neopterin levels were elevated in the sera of human immunodeficiency virus (HIV)-seronegative intravenous drug abusers and homosexuals, as well as in the sera of HIV-seropositive patients. Alpha IL-1 was the most predominant form of IL-1 found in the sera of all groups, and its level in HIV-seronegative intravenous drug abusers was elevated compared with the level in controls, whereas its levels in asymptomatic HIV-seropositive intravenous drug abusers and asymptomatic HIV-seronegative and HIV-seropositive homosexuals were decreased relative to the level in controls. Beta IL-1 levels in sera in all groups were not significantly different from the control value, except for the HIV-seropositive homosexual group; in this group the beta IL-1 level was significantly decreased compared with the control value.

Acquired immunodeficiency syndrome (AIDS) is caused by human immunodeficiency virus (HIV) infection and is primarily a disease of the immune system (4). Characteristic laboratory features of AIDS include lymphopenia, a decreased number of CD4 lymphocytes, and reversal of the ratio of CD4 (helper/inducer) to CD8 (suppressor/cytotoxic) lymphocytes (8). In addition, patients with AIDS-related complex or AIDS have been reported to have increased levels of soluble interleukin-2 (IL-2) receptors, soluble CD8, β_2 -microglobulin, lysozyme, acid-labile human leukocyte interferon, and thymosin α in serum (1, 11, 15).

Neopterin (6-D-erythro-trihydroxypropylpterin) is a low-molecular-weight compound derived from GTP (5). The cytokine IL-1 occurs as two biochemically distinct proteins, namely IL-1 alpha and IL-1 beta (2). Each IL-1 is encoded by a separate gene. These two forms share 26% amino acid sequences, bind the same receptor on target cells, and exhibit an identical broad spectrum of biological activities on a variety of cells (2, 7, 12, 18). The monocyte-macrophage is an important source of neopterin, IL-1 alpha, and IL-1 beta (2, 5, 12, 18). Monocytes play a major role in the propagation and pathogenesis of HIV infection, since certain subsets of monocytes express CD4 antigen and monocyte function is reported to be abnormal in patients with AIDS (4, 13, 14, 17); therefore, we have measured neopterin, IL-1 alpha, and IL-1 beta levels in the sera of HIV-seronegative and HIV-seropositive intravenous drug abusers (IVDA) and homosexuals and patients with lymphadenopathy or AIDS.

MATERIALS AND METHODS

Subjects. The patients included 60 HIV-seronegative and 44 HIV-seropositive asymptomatic IVDA, 40 HIV-seronegative and 40 HIV-seropositive homosexuals, 70 patients with uncomplicated generalized lymphadenopathy, and 89 patients with conditions meeting the Centers for Disease Control surveillance definition of AIDS. Of these patients, 47 had *Pneumocystis carinii* pneumonia, 27 had Kaposi's sar-

coma, and 15 had both *P. carinii* pneumonia and Kaposi's sarcoma. Also included in the study were 26 normal control subjects who were HIV seronegative.

Methods. The serum samples obtained were stored at -70°C until assayed.

(i) **Neopterin assay.** Neopterin levels in serum were determined by the Henning Berlin neopterin assay (Henning Berlin GmbH, Berlin, Federal Republic of Germany), in which ^{125}I -labeled neopterin and a specific antineopterin antiserum are used. The percentage of bound ^{125}I -labeled antigen decreases as a function of increasing concentrations of unlabeled antigen. The bound and free radiolabeled antigen were separated by precipitation of the antigen-antibody complexes with polyethylene glycol. The quantity of unlabeled antigen in an unknown sample was determined by comparing the radioactivity of the precipitate after centrifugation with that of known standards. The neopterin levels were expressed in nanograms per milliliter. The coefficient of variation was 5%.

(ii) **IL-1 alpha assay.** IL-1 alpha was quantitated by using an IL-1 alpha enzyme-linked immunosorbent assay (Endogen, Inc., Boston, Mass.). Standards and serum samples were incubated in specific monoclonal antibody-linked IL-1 alpha-coated microdilution plate wells. After the samples had been washed, rabbit antibody to IL-1 alpha was added, and the mixture was incubated. The samples were washed again, and a third antibody (alkaline phosphatase-conjugated goat anti-rabbit antibody) was added. Then, the mixture was washed, the substrate was added, and the color change was measured at 405 nm by using an enzyme-linked immunosorbent assay reader. Unknown values were calculated from a standard curve obtained by plotting the dilutions of the IL-1 alpha standard against A_{405} and were expressed in picograms per milliliter. The coefficient of variation was 7%.

(iii) **IL-1 beta assay.** IL-1 beta was quantitated by using the Cistron IL-1 beta ELISA kit (Cistron Biotechnology, Inc., Pine Brook, N. J.). Serum samples and standards were added to microdilution plate wells coated with monoclonal antibody specific for IL-1 beta and incubated. Then, polyclonal rabbit anti-IL-1 beta was added to the wells. After

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TABLE 1. Neopterin, IL-1 alpha, and IL-1 beta levels in control and study groups

Group	No. of subjects	HIV antibody status	Mean level (± SE) of:		
			Neopterin (ng/ml)	IL-1 alpha (pg/ml)	IL-1 beta (pg/ml)
Normal controls	26	Negative	1.19 ± 0.12	359 ± 146	4.04 ± 1.4
Asymptomatic IVDA	60	Negative	1.75 ± 0.21 (<0.05 ^a)	581 ± 44 (<0.05 ^a)	7.71 ± 2.33 (NS ^a)
Asymptomatic IVDA	44	Positive	3.94 ± 0.57 (<0.0005, ^a <0.0005 ^b)	109 ± 17 (<0.025, ^a <0.0005 ^b)	6.70 ± 2.80 (NS, ^a NS ^b)
Asymptomatic homosexuals	40	Negative	1.76 ± 0.14 (<0.005 ^a)	113 ± 8 (<0.025 ^a)	1.22 ± 0.76 (<0.025 ^a)
Asymptomatic homosexuals	40	Positive	3.05 ± 0.20 (<0.0005, ^a <0.0005 ^c)	83 ± 4 (<0.025, ^a <0.0025 ^c)	1.76 ± 1.13 (NS, ^a NS ^c)
Lymphadenopathy patients	70	Positive	3.61 ± 0.28 (<0.0005 ^a)	203 ± 33 (NS ^a)	6.14 ± 2.60 (NS ^a)
AIDS patients	89	Positive	18.60 ± 2.10 (<0.0005 ^a)	236 ± 39 (NS ^a)	42.50 ± 15.08 (NS ^a)

^a P value compared with normal controls (Student's *t* test); NS, not significant.
^b P value compared with HIV-seronegative IVDA; NS, not significant.
^c P value compared with HIV-seronegative homosexuals; NS, not significant.

further incubation, goat anti-rabbit immunoglobulin G conjugated to horseradish peroxidase enzyme was added. After each addition, the wells were washed. Finally, the enzyme substrate was added to the wells and the color intensity was

measured with an enzyme-linked immunosorbent assay reader at 490 nm. Unknown values were obtained from a standard curve and expressed in picograms per milliliter. The coefficient of variation was 13%.

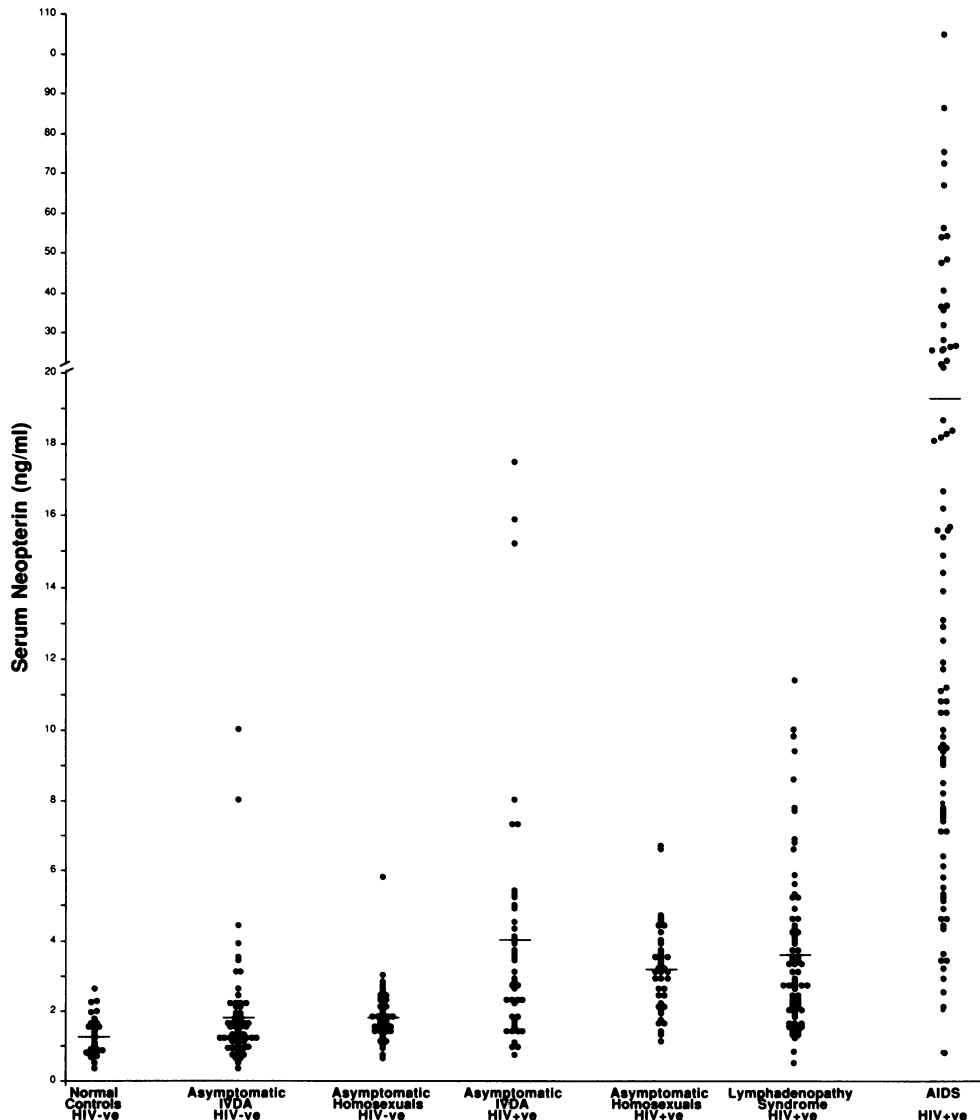


FIG. 1. Neopterin levels in sera of asymptomatic IVDA, homosexuals, and patients with lymphadenopathy or AIDS.

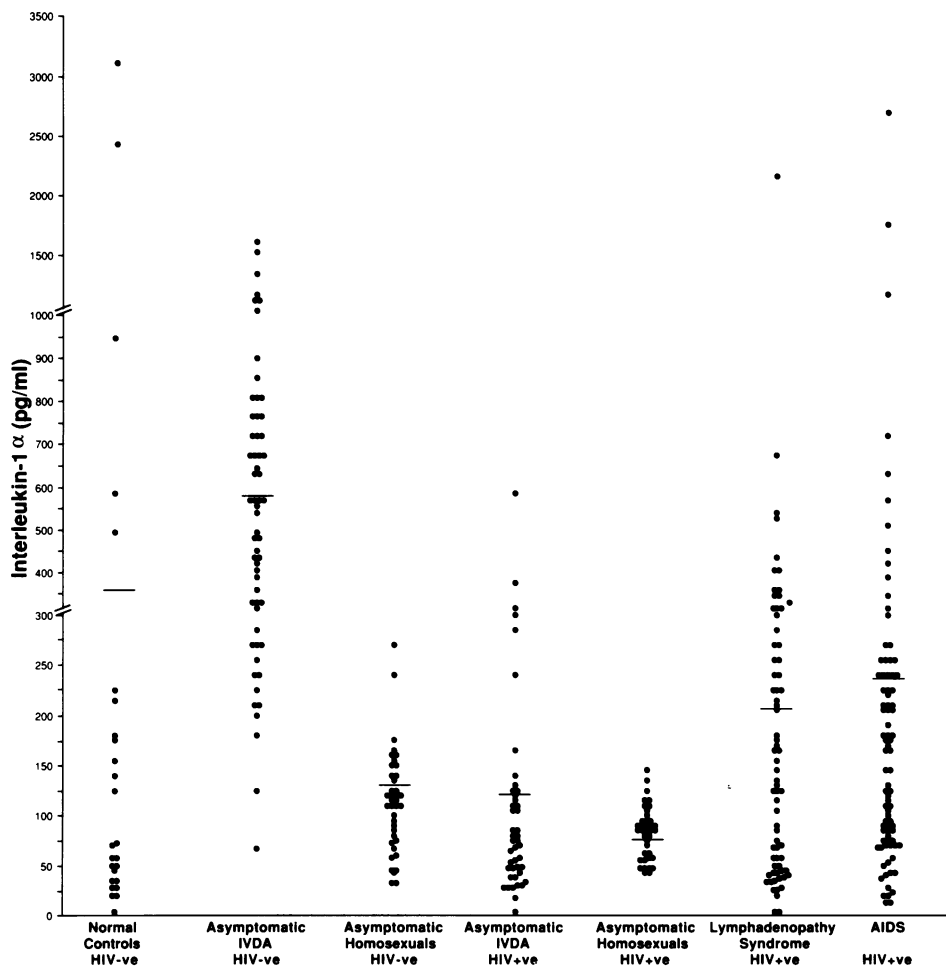


FIG. 2. IL-1 alpha levels in sera of asymptomatic IVDA, homosexuals, and patients with lymphadenopathy or AIDS.

RESULTS

Neopterin values in normal controls, IVDA, homosexuals, and patients with lymphadenopathy or AIDS are given in Table 1 and depicted in Fig. 1. The mean neopterin level in the sera of normal controls was 1.19 ± 0.12 ng/ml (mean \pm standard error of the mean) compared with 1.75 ± 0.21 ng/ml in the sera of HIV-seronegative IVDA and 1.76 ± 0.14 ng/ml in the sera of HIV-seronegative homosexuals. This increase in the mean neopterin levels in the sera of HIV-seronegative IVDA and HIV-seronegative homosexuals is statistically significant. Similar statistically significant increases were observed in the sera of HIV-seropositive IVDA, homosexuals, and patients with lymphadenopathy. The mean neopterin level in the sera of patients with AIDS was 18.6 ± 2.10 ng/ml, and this increase is highly significant.

IL-1 alpha levels in the sera of normal controls and HIV-seronegative and HIV-seropositive groups are shown in Table 1 and depicted in Fig. 2. The mean IL-1 alpha level in the sera of normal controls was 359 ± 146 pg/ml compared with 581 ± 44 pg/ml in the sera of HIV-seronegative IVDA. This increase is statistically significant. IL-1 alpha levels in the sera of HIV-seropositive IVDA and HIV-seropositive and HIV-seronegative homosexuals were decreased significantly compared with control values, whereas IL-1 alpha levels in the sera of patients with lymphadenopathy or AIDS were not significantly different from control values.

IL-1 beta levels in the sera of HIV-seronegative and HIV-seropositive groups are given in Table 1 and shown in Fig. 3. The mean IL-1 beta levels for all groups were not significantly different from control values, except for the HIV-seronegative homosexual group; for this group, the level of IL-1 beta in serum was significantly lower than the control value. Whereas there were measurable levels of IL-1 beta in the sera of 42% of controls, the levels in the patient groups were 34% for HIV-seronegative asymptomatic IVDA, 39% for HIV-seropositive IVDA, 8% for HIV-seronegative homosexuals, 11% for HIV-seropositive homosexuals, 16% for patients with lymphadenopathy, and 63% for patients with AIDS.

Both HIV-seronegative and HIV-seropositive subjects had much higher levels of IL-1 alpha than IL-1 beta in their circulations.

DISCUSSION

The primary immunologic defect in subjects infected with HIV is depletion of the CD4 (helper/inducer) lymphocyte subset, leading to lymphopenia, decreased CD4/CD8 ratios, and decreased T-cell and B-cell function (4, 8). Other laboratory parameters which increase in HIV-infected persons include β_2 -microglobulin levels in serum and urine, soluble IL-2 receptors, HIV P24 antigen levels in serum, acid-labile alpha interferon levels in serum, thymosin α and thymosin β_4

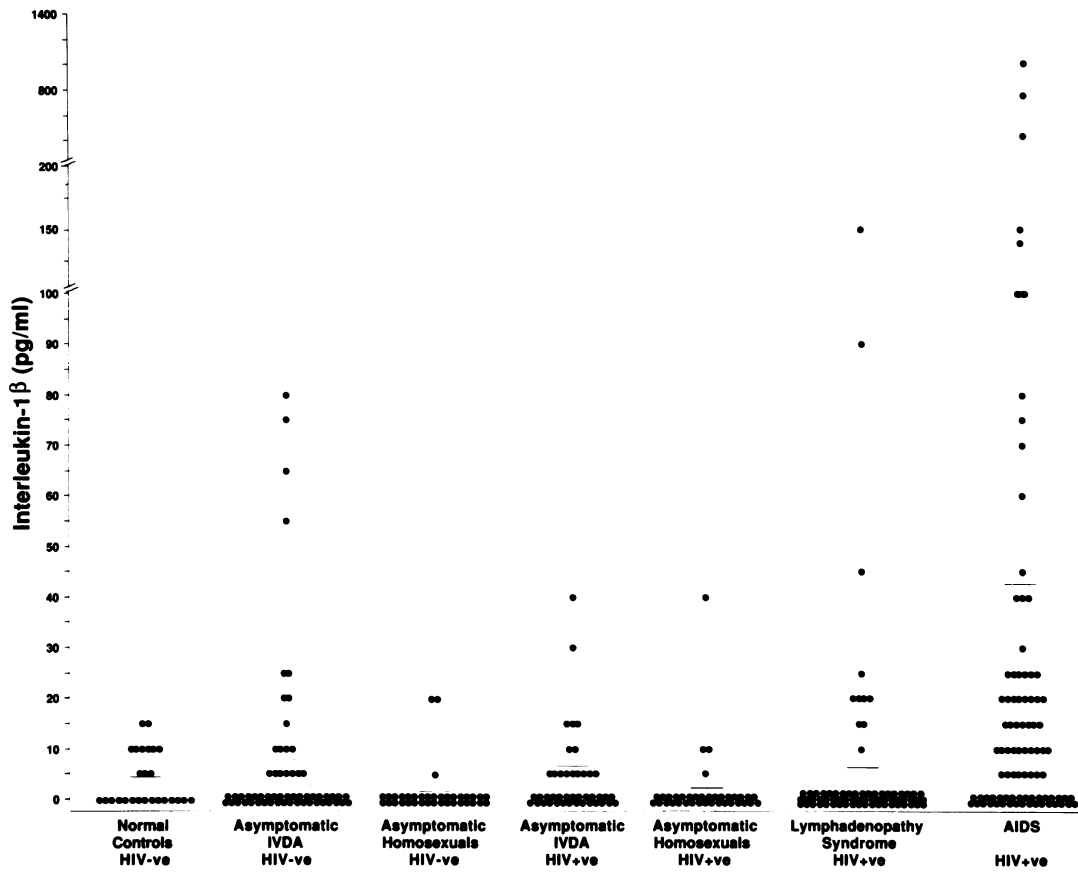


FIG. 3. IL-1 beta levels in sera of asymptomatic IVDA, homosexuals, and patients with lymphadenopathy or AIDS.

levels in serum, and soluble CD8 and neopterin levels in serum and urine (1, 5, 8, 11, 15, 16).

The results of the present study demonstrate that the neopterin levels in serum increase in subjects in groups at risk for HIV infection as well as in patients infected with HIV. However, monocyte function is defective in patients with AIDS both in random locomotion and chemotaxis and in phagocytosis (13, 14, 17), although Murray et al. (10) reported that the antimicrobial function of monocytes in AIDS patients was intact and could be stimulated with gamma interferon. Neopterin levels are elevated in the serum and urine of patients with AIDS-related complex and AIDS (5, 6, 8), and neopterin has recently been suggested as one of four laboratory markers that may be used to predict which patients will progress to AIDS after HIV infection (9). Fuchs et al. (6) suggested that high neopterin levels might indicate a high risk for the development of progressive HIV disease. Our results suggest that HIV-seronegative as well as HIV-seropositive IVDA and homosexuals also have increased levels of neopterin compared with normal controls. The abnormally elevated neopterin levels in the sera of HIV-seronegative IVDA and homosexuals may be due to the variety of infections that these individuals contract, such as hepatitis B and Epstein-Barr virus and cytomegalovirus infections (3). These infections in addition to HIV infection probably cause a significant increase of neopterin levels in HIV-seropositive patients. Elevated neopterin levels in cancer patients and in patients with viral infections have been reported, and it has been speculated that high neopterin levels might reflect the response of the body to tumor cells

and virally transformed cells (5). Neopterin is produced by macrophages after stimulation by gamma interferon, which is in turn produced by activated T lymphocytes (5). Chronic exposure to and reinfection by this variety of infectious agents could lead to a persistently elevated level of activated T cells, which, in turn, could produce elevated levels of gamma interferon. Gamma interferon may in turn cause elevated production of neopterin by macrophages.

Despite the amino acid difference in their primary sequences, IL-1 alpha and beta modulate a variety of cellular actions leading to various events associated with inflammation and immunologic reactions (2, 12, 18). IL-1 has been reported to modulate the proliferation, maturation, and biologic activity of T lymphocytes, B lymphocytes, and other tissue cells (12). Circulating IL-1 alpha levels were much higher than circulating IL-1 beta levels, and IL-1 alpha appears to be the predominant form in the circulation. Although all but four subjects (99%) produced detectable levels of IL-1 alpha, only 34% of the subjects produced detectable levels of IL-1 beta in their circulation. The predominance of IL-1 alpha in serum might be due to preferential synthesis of IL-1 alpha over IL-1 beta by some cell types.

Circulating levels of IL-1 have been reported to increase during some infections and inflammatory diseases, whereas decreased production has been observed in patients with metastatic tumors and nutritional deficiencies (2). Increased circulating levels of IL-1 alpha were observed in our HIV-seronegative IVDA. This increase might be due to chronic exposure to a variety of infections such as cytomegalovirus

and Epstein-Barr virus infections (3). However, IL-1 alpha levels were decreased in the HIV-seropositive IVDA and HIV-seronegative and HIV-seropositive homosexual groups of patients, although these groups also are constantly exposed to these infectious organisms; the reason for this is unclear. At present, there is no clear evidence to indicate that diseases are caused by primary IL-1 deficiency or overproduction. IL-1 alpha can help the body eliminate noxious agents or injurious processes of an endogenous origin and also can become a mediator of tissue injury (18). A low dose of recombinant IL-1 enhanced the resistance of both normal and granulocytopenic mice to infection with *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (19), suggesting a possible use for IL-1 in the treatment of opportunistic infections in patients with AIDS.

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