JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1989, p. 257–260
0095-1137/89/020257-04$02.00/0

Vol. 27, No. 2

Elevated Soluble CD8 Levels in Sera of Human Immunodeficiency Virus-Infected Populations

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Received 1 August 1988/Accepted 25 October 1988

Soluble CD8 levels in sera were quantitated in asymptomatic intravenous drug abusers, homosexuals, and patients with lymphadenopathy or acquired immunodeficiency syndrome. Soluble CD8 levels were elevated in human immunodeficiency virus-seronegative intravenous drug abusers and homosexuals, probably reflecting infections like cytomegalovirus. The sera of human immunodeficiency virus-seropositive groups of patients with human immunodeficiency virus infection also had elevated levels of soluble CD8, reflecting infections like cytomegalovirus and human immunodeficiency virus infection.

The CD4 and CD8 glycoproteins, which are expressed on the surface of subsets of T lymphocytes, belong to the immunoglobulin gene superfamily (23). Human T lymphocytes are classified as helper-inducer (CD4) and suppressor-cytotoxic (CD8) cell subgroups according to the cell surface phenotypes (19). Whereas CD4 is a 55-kilodalton monomer, the 32-kilodalton CD8 molecule forms disulfide-linked homodimers on the surface of peripheral T lymphocytes (13). Expression of the CD4 and CD8 molecules correlates with the specificity of T cells for either class II or class I major histocompatibility complex molecules on target cells (20).

The human immunodeficiency virus (HIV) has been identified as the etiologic agent of acquired immune deficiency syndrome (AIDS) (8). Although CD4 acts as the receptor for the HIV retrovirus and HIV tropism is dependent on the expression of CD4 on target cells (13, 14), CD8 cells are elevated after HIV infection (3). Fujimoto and his colleagues (9, 10) reported that supernatants of $CD8^+$ human leukemic cell lines and serum from patients with $CD8^+$ T-cell leukemias contained high levels of immunoreactive soluble CD8 (SCD8) glycoprotein. In this study we sought to determine whether the HIV infection had any effect on SCD8 levels.

MATERIALS AND METHODS

Subjects. The patients included 110 HIV-seronegative and 46 HIV-seropositive asymptomatic intravenous drug abusers (IVDA), 44 HIV-seronegative and 41 HIV-seropositive asymptomatic homosexuals, 39 patients with uncomplicated generalized lymphadenopathy, 48 patients with *Pneumocystis carinii* pneumonia, 27 patients with Kaposi's sarcoma, and 15 patients with both *P. carinii* pneumonia and Kaposi's sarcoma who met the Centers for Disease Control surveillance definition of AIDS (2). Thirty normal control subjects who were HIV seronegative were also included in the study.

CD4 and CD8 lymphocytes. Samples (10 ml) of peripheral blood were drawn in syringes containing 100 U of heparin (Sigma Chemical Co., St. Louis, Mo.), after informed consent was obtained. Mononuclear cells were separated by Ficoll-Hypaque (Pharmacia Fire Chemicals, Piscataway, N.J.) centrifugation. CD4 and CD8 cells were enumerated by utilizing CD4 and CD8 antisera (Ortho Pharmaceutical Co., Raritan, N.J.) by indirect immunofluorescence (11). Absolute lymphocyte values were obtained from leukocyte counts and differential counts.

SCD8 assay. Sera obtained from the subjects were frozen at −70°C until analyzed. SCD8 levels in serum were determined by using a Cell Free CD8 test kit (T Cell Sciences, Inc., Cambridge, Mass.). This enzyme immunoassay developed by T Cell Sciences has been used to quantitate cell-free human T-cell CD8-like molecules in serum of patients with leukemias, allograft transplantation, and autoimmune diseases (M. Brown, S. Carrabis, S. Ip. and P. Kung, Fed. Proc. **46**:467, 1987; C.-H. Pui, S. H. Ip, R. K. Dodge, S. Carrabis, M. Brown, W. M. Crist, C. W. Berard, P. Kung, G. V. Dahl, and S. B. Murphy, Blood **70**[Suppl. 1]:207a, 1987). The standards or sera were added to the anti-CD8 monoclonal antibody-coated polystyrene microdilution plate wells. After incubation the wells were washed to remove unreactive sample components. An enzyme-conjugated anti-CD8 monoclonal antibody directed against a second epitope on the CD8 molecule was then added, which would bind to the CD8 captured by the first antibody. After removal of unbound enzyme-conjugated anti-CD8 by washing, substrate solution was added to the wells. The reaction was terminated by the addition of stop solution, and absorbance at 490 nm was measured with an enzyme-linked immunosorbent assay reader. A standard curve was prepared from five CD8 standards, and unknown values were determined from the standard curve and expressed as units per milliliter. The coefficients of variation (within run) for 18 replicate determinations were 3.4% for a control and 6.8 for a patient. The coefficient of variations (between run) were 5.5% for a control (five determinations) and 9.5% for a patient (five determinations).

Antibodies to HIV. An enzyme-linked immunosorbent assay and Western blots (immunoblots) against purified HIV proteins were performed at the National Cancer Institute as previously described (22).

RESULTS

The SCD8 level in the sera of 30 normal controls was 392 ± 33 U/ml (mean ± standard error) compared with 769 ± 45 U/ml in the sera of 110 HIV-seronegative asymptomatic IVDA and 643 ± 48 U/ml in the sera of 44 HIV-seronegative

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TABLE 1. Lymphocyte subsets and soluble CD8 levels in asymptomatic IVDA, homosexuals, and patients with lymphadenopathy or AIDS

Group	No. of subjects	HIV antibody	Lymphocytes per μl (mean ± SE)			SCD8 U/ml (mean 1 SE)
			Total	CD4	CD8	
Normal controls	30	Negative	1,994 ± 266	818 ± 137	440 ± 62	392 ± 33
Asymptomatic IVDA	110	Negative	2,568 ± 117	919 ± 46	625 ± 35	769 ± 45[a]
Asymptomatic IVDA	46	Positive	1,823 ± 109	485 ± 39	665 ± 52	1,512 ± 85[a,b]
Asymptomatic homosexuals	44	Negative	2,311 ± 109	653 ± 37	640 ± 36	643 ± 48[a]
Asymptomatic homosexuals	41	Positive	1,950 ± 134	543 ± 56	573 ± 48	1,114 ± 65[a,c]
Lymphadenopathy	39	Positive	1,882 ± 152	368 ± 40	769 ± 77	1,310 ± 90[a]
AIDS with *P. carinii* pneumonia	48	Positive	757 ± 84	129 ± 22	263 ± 32	1,301 ± 84[a]
AIDS with Kaposi's sarcoma	27	Positive	1,403 ± 150	298 ± 44	450 ± 44	1,231 ± 117[a]
AIDS with both *P. carinii* pneumonia and Kaposi's sarcoma	15	Positive	784 ± 104	137 ± 26	295 ± 54	1,362 ± 114[a]

[a] $P < 0.001$, compared with normal controls (Student's t test).
[b] $P < 0.001$ compared with HIV-seronegative IVDA.
[c] $P < 0.001$ compared with HIV-seronegative homosexuals.

homosexuals (Table 1). This increase in SCD8 in these seronegative groups is statistically significant ($P < 0.001$). The SCD8 level in the sera of HIV-seropositive asymptomatic IVDA was 1,512 ± 85 U/ml compared with 769 ± 45 U/ml in the sera of HIV-seronegative asymptomatic IVDA. This difference was statistically significant ($P < 0.001$). Similarly, the HIV-seropositive asymptomatic homosexual group had 1,114 ± 65 U/ml compared with 643 ± 48 U/ml in the HIV-seronegative asymptomatic homosexual group, which is also statistically significant ($P < 0.001$). Similar increases were observed in patients with lymphadenoathy and in patients with AIDS with *P. pneumonia*, Kaposi's sarcoma, or both (Fig. 1).

Total lymphocytes and CD4 lymphocytes decreased after HIV infection. On the other hand, absolute CD8 lymphocyte levels increased after HIV infection in asymptomatic patients. There was no correlation between CD8 lymphocytes and SCD8 levels.

DISCUSSION

HIV infection leads to a severe defect in cellular immunity which includes lymphopenia, decreased numbers of CD4 cells, increased numbers of CD8 cells, depressed CD4/CD8 cell ratios, decreased T-cell and B-cell function, anergy, and

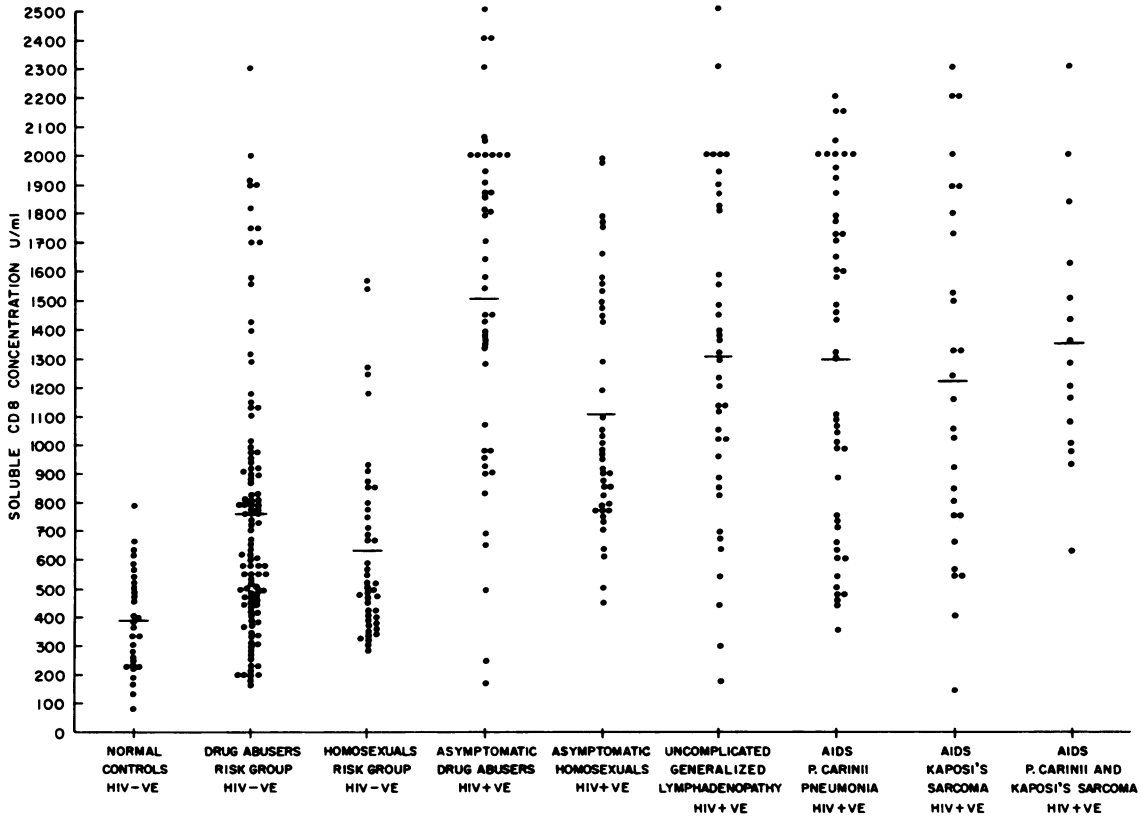

FIG. 1. Soluble CD8 levels in serum of asymptomatic IVDA, homosexuals, and patients with lymphadenopathy or AIDS.

defective monocyte function (8). In addition, increased beta 2-microglobulin in serum, lysozyme in serum, circulating acid-labile human leukocyte interferon, thymosin-alpha in serum, and soluble interleukin-2 receptor levels have been reported (4, 11, 15, 17, 18). The results of the present study demonstrate that SCD8 levels in serum were elevated in HIV-seropositive asymptomatic IVDA and homosexuals and in patients with lymphadenopathy or AIDS.

Our results showed that SCD8 levels in serum increased significantly in HIV-seropositive asymptomatic IVDA and homosexuals compared with HIV-seronegative IVDA and homosexuals. Absolute CD8 cells were also elevated in these groups, as has been reported previously (3, 12, 16). However, HIV-seronegative asymptomatic IVDA and homosexuals had significantly elevated SCD8 levels compared with the normal control group. The cause of abnormally elevated CD8 cells in many asymptomatic IVDA and homosexuals may be due to the variety of infections that these individuals contract such as infections with cytomegalovirus and Epstein-Barr virus (1, 5–7). The chronic exposure and reinfection with these agents could lead to a persistently elevated level of CD8 cells, since it has been shown that infections with such agents lead to a transient quantitative increase in the numbers of CD8 cells in the peripheral blood (1, 5–7). These infections that result in increased numbers of CD8 cells may result in elevated levels of SCD8 in the circulation.

Previous studies by Fujimoto et al. had demonstrated that supernatants of $CD8^+$ human leukemic cell lines as well as sera from patients with $CD8^+$ T-cell leukemias had elevated levels of SCD8 molecules (9, 10). They concluded that the soluble form of CD8 found in supernatants and serum resulted from a specific cleavage of the membrane-bound molecule, since a labeled 27-kilodalton form of CD8 was released from T cells after cell surface iodination. The released CD8 is 5 kilodaltons smaller than the membrane CD8 monomer, suggesting that proteolysis, alternative glycosylation, or alternative exon splicing may be responsible for the difference between the two molecules (13).

The amount of SCD8 has been shown to increase upon activation of CD8 in vitro (B. Tomkinson, M. C. Brown, S. Ip, and J. L. Sullivan, 2nd Annu. Conf. Clin. Immunol., 1987). It has been suggested that measurement of soluble CD8 may serve as an index of suppressor and cytotoxic activity. However, there was no correlation between the number of CD8 cells and SCD8 levels in our patients. Pui and his colleagues (Blood **70**[Suppl. 1]:207a) have shown that SCD8 molecules in serum were elevated in various pediatric lymphomas. Fujimoto and his colleagues (10) obtained evidence to indicate that the release of CD8 after antibody treatment was not due to cell destruction but was an antigen-specific phenomenon. Although the function of this released SCD8 molecule is unknown, CD8 lymphocytes may have a role in suppressing expression of HIV in vivo, as it has been shown that CD8 cells are able to control HIV infection in vitro (21). Further studies are needed to define the role of circulating SCD8 molecules in immune response to HIV infection and other viral diseases.

ACKNOWLEDGMENTS

We thank Stanley Weiss for performing enzyme-linked immunosorbent assays and Western blots and Mary Reilingh, Mark Sydlo, and Gary Jelich for providing excellent technical assistance.

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