β_2 -Microglobulin and Neopterin: Predictive Markers for Human Immunodeficiency Virus Type 1 Infection in Children?

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The value of β_2 -microglobulin and neopterin concentrations in serum for early diagnosis of infants born to human immunodeficiency virus type 1 (HIV-1)-infected mothers was assessed. Concentrations of both markers were measured in serum samples from pediatric patients (Centers for Disease Control classifications P0, P1, and P2), as well as in age-matched normal subjects. Both β_2 -microglobulin and neopterin were significantly increased in HIV-1-infected symptomatic subjects (P2) compared to controls. Seventy-five percent of asymptomatic patients (P1) also had increased values. On the other hand, a significant overlap in concentrations of both markers in serum was found between controls and P0 patients. Thirty-eight percent of the P0 patients had values comparable to those of the P2 group. Persistently high concentrations of both markers in P0 patients may be indicative of HIV-1 infection.

Human immunodeficiency virus type 1 (HIV-1) is the causative agent of the acquired immunodeficiency syndrome (AIDS). The primary pathogenic process is the progressive depletion of the CD4 (helper-inducer) lymphocyte subset which eventually leads to immune dysfunction (18, 20). Decreased numbers of CD4 T lymphocytes in HIV-1-infected adults are a useful predictor of disease progression to AIDS (6, 10, 11). Other laboratory markers of disease progression include p24 antigenemia (22, 28), increased serum concentrations of β_2 -microglobulin (β_2m) (11, 16, 22, 33, 34), and increased neopterin concentrations in serum or urine (3, 11, 14, 15). On the other hand, markers such as soluble interleukin-2 receptors (24, 26), soluble CD8 (1, 27), thymosin (23), and acid-labile interferon (8) are increased in HIV-1-infected subjects but are not useful in differentiating the various stages of the disease.

HIV-1 serology is not a useful test for diagnosis of infection in children who are offspring of infected mothers and younger than 15 months of age, primarily because of passively acquired maternal antibodies (12, 25, 32). The detection rate of p24 antigen in asymptotically infected children under 6 months of age was only 25%, and no p24 antigen was found in infected infants tested at birth (4). Formation of immune complexes between p24 antigen and maternally derived antibodies is the probable explanation for the low detection rates. Viral cultures are expensive and laborious and require too much blood to be useful for neonates (17). CD4 T-lymphocyte numbers usually do not decline to abnormally low values in children until advanced stages of the disease are reached (12).

The rate of maternal-child transmission of infection has been estimated at 29 to 73% by various investigators, indicating that 27 to 71% of seropositive infants are not infected (2, 12, 21, 31). To provide early treatment of infection, it is essential to achieve an early diagnosis. In the present study, we investigated the clinical utility of β_2m and neopterin concentrations in serum as early indicators of HIV-1 infec-

tion in infants and children born of infected mothers. These two surrogate markers are byproducts of immune activation and cellular activity and have proven predictive value in infected adults.

MATERIALS AND METHODS

Subjects. The study population consisted of 83 HIV-1-seropositive infants and children, aged 2 months to 9 years. Patients were divided into three groups according to the Centers for Disease Control (CDC) surveillance definitions (7). Forty-five patients under the age of 15 months (mean $\pm$ standard deviation, 7 ± 3.4 months) with indeterminate HIV-1 status were classified as P0, 16 asymptomatic patients (30.7 ± 20.7 months) were classified as P1, and 22 symptomatic patients (36.3 ± 33.3 months) were classified as P2. Nine of the latter group had *Pneumocystis carinii* pneumonia, one exhibited progressive neurologic disease, and twelve suffered from lymphocytic interstitial pneumonitis and recurrent bacterial infections. Included in this study were 76 HIV-1-seronegative controls. The control subjects could be subdivided into five age groups: 13 were 2 to 12 months (7 ± 3.4 months), 11 were 13 to 24 months (16.7 ± 3.0 months), 12 were 25 to 36 months (31.6 ± 4.4 months), 20 were 4 to 6 years (62.3 ± 9.1 months), and the remaining 20 were 7 to 9 years (97.1 ± 8.6 months). The mean age of the controls taken as a group was 47.8 ± 34.5 months.

Methods. Sera were obtained from patients and controls and stored at -70°C until being assayed. Peripheral blood was collected in EDTA-containing tubes. Lymphocytes were selectively recovered by using a Ficoll-Hypaque density gradient (5). Percentages of CD4 and CD8 T-lymphocyte subpopulations were quantitated by indirect immunofluorescence after reaction with mouse monoclonal antibodies to T4 and T8 antigens (Coulter Diagnostics, Hialeah, Fla.), followed by washing and reaction with fluorescein-conjugated anti-mouse immunoglobulin serum (Organon Teknika-Cappel, Malvern, Pa.).

Antibody to HIV-1 was detected by enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago,

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TABLE 1. β_2 m and neopterin concentrations in serum, percentage of CD4 lymphocytes, CD4/CD8 ratio, and p24 antigenemia in control and study groups

Group	HIV-1 antibody status	Concn in serum [mean $\pm$ SD (no. tested)]		% CD4 lymphocytes [mean $\pm$ SD (no. tested)]	CD4/CD8 ratio [mean $\pm$ SD (no. tested)]	No. exhibiting p24 antigenemia/no. tested
		β_2 m (mg/liter)	Neopterin (ng/ml)			
Control Patients	Negative	1.2 $\pm$ 0.5 (76)	1.1 $\pm$ 0.4 (27)	47.6 $\pm$ 5.4 (20)	2.0 $\pm$ 0.3 (20)	0/0
CDC P0	Positive	2.1 $\pm$ 1.4 (45) ^{a,b,c}	1.9 $\pm$ 1.3 (31) ^b	47.2 $\pm$ 7.5 (45) ^{b,c}	1.9 $\pm$ 0.6 (45) ^{b,c}	0/45
CDC P1	Positive	2.7 $\pm$ 0.9 (16) ^a	3.1 $\pm$ 1.7 (10) ^a	37.0 $\pm$ 8.1 (16) ^a	1.2 $\pm$ 0.4 (16) ^a	6/16
CDC P2	Positive	4.1 $\pm$ 1.8 (22) ^a	5.5 $\pm$ 2.8 (19) ^a	30.8 $\pm$ 7.1 (22) ^a	0.9 $\pm$ 0.4 (22) ^a	15/22

^a $P < 0.001$, compared with normal controls (Student's t test).^b $P < 0.001$, compared with CDC P1.^c $P < 0.001$, compared with CDC P2.

Ill.) and confirmed by Western blot (immunoblot) (E. I. Du Pont de Nemours & Co., Inc., Wilmington, Del.). HIV-1 antigen was detected by solid-phase antigen capture enzyme-linked immunosorbent assay (Abbott Laboratories). The specificity of positive results was verified by the manufacturer's antigen neutralization assay. A ratio of <0.5 between the optical densities of neutralized and unneutralized sera was considered confirmatory.

β_2 m in serum was determined by immunoturbidimetry using antisera to β_2 m from Boehringer Mannheim Biochemicals (Indianapolis, Ind.) and calibrators from Sigma Chemical Co., (St. Louis, Mo.) on the Cobas-MIRA Analyzer (Roche Analytical Instruments, Nutley, N.J.) (29). Neopterin in serum was measured by radioimmunoassay (Neopterin-RIA; Henning-Berlin, Berlin, Federal Republic of Germany).

Statistical analysis. The unpaired Student's t test and linear regression were used in the statistical analysis. The level of significance was established at $P < 0.05$.

RESULTS

Mean serum β_2 m and neopterin results are shown in Table 1. All seronegative control subjects regardless of the age ranges had low concentrations of both serum markers (mean, 1.2 $\pm$ 0.5 mg/liter and 1.1 $\pm$ 0.4 ng/ml for β_2 m and neopterin, respectively). In contrast, significantly higher concentrations of both markers were observed in serum samples from seropositive patients. β_2 m concentrations progressively increased from 2.1 $\pm$ 1.4 mg/liter to 4.1 $\pm$ 1.8 mg/liter for CDC groups P0, P1, and P2. A similar trend was observed for neopterin in serum (mean, 1.9 $\pm$ 1.3 ng/ml for P0 and 5.5 $\pm$ 2.8 ng/ml for P2). The CD4/CD8 ratio and percentage of CD4 cells were determined for all 83 patients and 20 controls. There was no difference between patients in the P0 group and the control subjects. With advanced disease, the mean CD4/CD8 ratio and CD4 percentage significantly decreased from 1.9 $\pm$ 0.6 to 0.9 $\pm$ 0.4 and 47.2% $\pm$ 7.2% to 30.8% $\pm$ 7.1%, respectively. HIV-1 antigen was

FIG. 1. Comparison of β_2 m and neopterin concentrations in serum samples from controls and HIV-1-seropositive pediatric patients (CDC classifications P0, P1, and P2).

FIG. 2. β_2 m and neopterin concentrations in serum samples from controls and HIV-1-seropositive pediatric patients (CDC classifications P0, P1, and P2). *ARC, AIDS-related complex.

not detected in serum samples from all infected patients. Only 6 of 16 P1 patients and 15 of 22 P2 patients were antigenemic.

Figure 1 shows the relationship between β_2 m and neopterin concentrations in serum samples from the control and patient groups. Both markers were highly correlated ($r = 0.81$, $P < 0.001$). Concentrations of β_2 m and neopterin in serum from each study subject are depicted in Fig. 2. There was a significant overlap in concentrations of both markers between serum samples from control patients and serum samples from patients in the P0 group. For β_2 m, 62% (28 of 45) of P0 patients had values below 2 mg/liter whereas the remainder had values comparable to those of the P2 group. Surprisingly, 25% (4 of 16) of patients in the P1 group had values similar to those of the control group. Equivalent findings were obtained for concentrations of neopterin in serum. The specificity and sensitivity of both serum markers were calculated on the assumption that control subjects were uninfected and P1 and P2 patients were infected. Both

markers revealed similar specificity and sensitivity (β_2 m: specificity, 95%, sensitivity, 89%; neopterin: specificity, 96%, sensitivity, 86%).

DISCUSSION

β_2 m is a subunit of the human leukocyte class I antigen and is present on the surface of all nucleated cells. Under unstimulated conditions, the cellular source for most β_2 m in serum is B lymphocytes. Upon immune-system activation, both T and B lymphocytes actively release β_2 m into the circulation. Neopterin, on the other hand, is produced and released predominantly by macrophages after these cells have been stimulated by gamma interferon. B lymphocytes also produce neopterin, but to a lesser extent. Other lymphokines such as tumor necrosis factor and interleukin-2 enhance production of both markers. Recent evidence showed that inactivated HIV-1 alone directly enhances neopterin production but not β_2 m in an in vitro assay system

(B. Hofmann, C. Uittenbogaart, P. Nishanian, A. Rezai, and J. L. Fahey, Abstr. Fourth Annu. Conf. Clin. Immunol. 1989, abstr. no. 91). This observation may explain the increased neopterin concentrations found in serum samples from HIV-1-infected individuals.

Results of the present study demonstrate that β_2m and neopterin values are significantly increased in serum samples from children infected with HIV-1. This finding is consistent with observations made for adults. Our main interest lies in newborn children assigned to the P0 group because of maternal seropositivity. Circulating maternal antibodies may persist for 15 months and hamper serologic evaluation of these children. Thus, the HIV-1 status of these children is uncertain and administration of potentially toxic chemotherapy may be difficult to justify. Other adult markers of infection such as p24 antigenemia and CD4 cell counts are not very informative in newborn children, probably because of the presence of maternal p24 antibody, low numbers of infected cells, and high lymphocyte counts.

If we were to categorize our P0 children according to β_2m and neopterin concentrations in their serum samples, they could be divided into two groups: one with increased concentrations similar to those found for our P1 and P2 subjects and the other with low values similar to those of our controls. Assuming that increased production of both markers is indicative of immune activation by the virus, persistently low values suggest lack of infection. However, increased concentrations are not necessarily caused by HIV-1 infection because Epstein-Barr virus, hepatitis B virus, and cytomegalovirus infections can also induce increased production of both markers (13; J. L. Sullivan, B. Hofmann, and J. L. Fahey, Abstr. Fourth Annu. Conf. Clin. Immunol. 1989, abstr. no. 30). It is vital to rule out other infections when confronted with increased β_2m and neopterin values. Both markers should remain elevated during longitudinal studies if a child is infected with HIV-1.

Recently, polymerase chain reaction (PCR) amplification of HIV-1 proviral DNA in infected lymphocytes was used by several investigators for early diagnosis of infection in infants and children. The sensitivity and specificity of PCR were >90 and 100%, respectively, for infants older than 2 months. However, the sensitivity was only 54% for infants aged 1 to 16 days (9, 19, 32). Since PCR is a relatively new test and cross-contamination has been reported as a problem, it is important to confirm PCR-diagnosed infections with other test modalities (30). An attractive combination that should improve the efficiency of early diagnosis in infants would be PCR testing of lymphocytes followed by confirmatory testing of serum samples of positive patients for concentrations of β_2m and/or neopterin.

On the basis of our present findings, β_2m and neopterin concentrations in serum help discriminate between HIV-1-infected and uninfected children. Determining the clinical usefulness of these markers for early diagnosis of infection in P0 infants will require longitudinal studies of this patient population.

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LITERATURE CITED

- Agostinus, C., G. Semenzato, F. Venante, A. Sinicco, L. Trentin, R. Zambello, B. Zuppin, R. Zanotti, F. Swiero, D. Veneri, R. Foa, and G. Pizzolo. 1989. Increased levels of soluble CD8 molecule in the serum of patients with acquired immunodeficiency syndrome (AIDS) and AIDS related disorders. *Clin. Immunol. Immunopathol.* **50**:146-153.
- Blanche, S., F. Le Deist, A. Fischer, F. Veber, M. Debre, S. Chamaret, L. Montagnier, and C. Griscelli. 1986. Longitudinal study of 18 children with perinatal LAV/HTLV III infection: attempt at prognostic evaluation. *J. Pediatr.* **109**:965-970.
- Bogner, J. R., A. Matuschke, B. Heinrich, E. Eberle, and F. D. Goebel. 1988. Serum neopterin levels as predictor of AIDS. *Klin. Wochenschr.* **66**:1015-1018.
- Borkowsky, W., K. Krasinsky, D. Paul, R. Holzman, T. Moore, D. Bebenroth, R. Lawrence, and S. Chandwani. 1989. Human immunodeficiency virus type I antigenemia in children. *J. Pediatr.* **114**:940-945.
- Boyum, A. 1968. Isolation of leukocytes from human blood. Further observations—methyl, cellulose, dextran and ficoll as erythrocyte aggregating agents. *Scand. J. Clin. Invest. Suppl.* **97**:31-49.
- Cavaille-Coll, M., A. Messiah, D. Klatzmann, W. Rozenbaum, D. Lachuvir, S. Kernbaum, E. Brisson, F. Chapuis, C. Blanc, P. Debre, and J. C. Gluckman. 1984. Critical analysis of T cell subset and function evaluation in patients with persistent generalized lymphadenopathy in groups at risk for AIDS. *Clin. Exp. Immunol.* **57**:511-519.
- Centers for Disease Control. 1988. Classification system for human immunodeficiency virus (HIV) infection in children under 13 years of age. *Morbidity and Mortality Weekly Rep.* **36**:225-230.
- DeStefano, E., R. M. Friedman, A. E. Friedman-Kien, J. J. Goedert, D. Henriksen, O. T. Preble, J. A. Sonnabend, and K. J. Vilcek. 1982. Acid-labile human leukocyte interferon in homosexual men with Kaposi's sarcoma and lymphadenopathy. *J. Infect. Dis.* **146**:451-455.
- Edwards, J. R., P. P. Ulrich, P. S. Weintrub, M. J. Cowan, J. A. Levy, D. W. Wara, and G. N. Vyas. 1989. Polymerase chain reaction compared with concurrent viral cultures for rapid identification of human immunodeficiency virus infection among high-risk infants and children. *J. Pediatr.* **115**:200-203.
- Fahey, J. L., H. Prince, M. Weaver, J. Groopman, B. Visscher, K. Schwartz, and R. Detels. 1984. Quantitative changes in T helper or T suppressor/cytotoxic subsets that distinguish acquired immunodeficiency syndrome from other immune subset disorders. *Am. J. Med.* **76**:95-100.
- Fahey, J. L., J. M. G. Taylor, R. Detels, B. Hofmann, R. Melmed, P. Nishanian, and J. V. Giorgi. 1990. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type I. *N. Engl. J. Med.* **322**:166-172.
- Falloon, J., J. Eddy, L. Wiener, and P. A. Pizzo. 1989. Human immunodeficiency virus infection in children. *J. Pediatr.* **114**:1-30.
- Fauci, A. S. 1985. Immunologic abnormalities in the acquired immunodeficiency syndrome (AIDS). *Clin. Res.* **32**:491-499.
- Fuchs, D., A. Hausen, G. Reibnegger, H. Reissigl, D. Schonitzer, T. Spira, and H. Wachter. 1984. Urinary neopterin in the diagnosis of acquired immune deficiency syndrome. *Eur. J. Clin. Microbiol.* **3**:70-71.
- Fuchs, D., A. Hausen, G. Reibnegger, E. R. Werner, M. P. Dierich, and H. Wachter. 1988. Neopterin as a marker for activated cell-mediated immunity: application in HIV infection. *Immunol. Today* **9**:150-155.
- Grieco, M. H., M. M. Reddy, H. B. Kothari, M. Lange, E. Buimovici-Klein, and D. William. 1984. Elevated β_2 -microglobulin and lysozyme levels in patients with acquired immune deficiency syndrome. *Clin. Immunol. Immunopathol.* **32**:174-184.
- Jackson, J. B., and H. H. Balorin. 1988. Practical diagnostic testing for human immunodeficiency virus. *Clin. Microbiol. Rev.* **1**:124-138.
- Lane, H. C., and A. S. Fauci. 1985. Immunologic abnormalities in the acquired immunodeficiency syndrome. *Annu. Rev. Immunol.* **3**:477-492.
- Laure, F., V. Courgnaud, C. Rouzioux, S. Blanche, F. Veber, M. Burgard, C. Jacomet, C. Griscelli, and C. Brechot. 1988. Detection of HIV1 DNA in infants and children by means of the

- polymerase chain reaction. *Lancet* **ii**:538-541.
20. Lifson, A. R., G. W. Rutherford, and H. W. Jaffe. 1988. The natural history of human immunodeficiency virus infection. *J. Infect. Dis.* **158**:1360-1367.
 21. Mok, J. Q., C. Giaquinto, and A. DeRossi. 1987. Infants born to mothers seropositive for human immunodeficiency virus. *Lancet* **i**:1164-1168.
 22. Moss, A. R., P. Bacchetti, D. Osmond, W. Krampf, R. E. Chaisnon, D. Stites, J. W. Iber, J. P. Allain, and J. Carlson. 1988. Seropositivity for HIV and the development of AIDS or AIDS related condition: three year follow up of the San Francisco General Hospital cohort. *Br. Med. J.* **296**:745-750.
 23. Naylor, P. H., A. Friedman-Kien, E. Hersh, M. Erdo, and A. L. Goldstein. 1986. Thymosin alpha-1 and thymosin beta-4 in serum: comparison of normal, cord, homosexual, and AIDS serum. *Int. J. Immunopharmacol.* **8**:667-676.
 24. Prince, H. E., S. Kleinman, and A. E. Williams. 1988. Soluble IL-2 receptor levels in serum from blood donors seropositive for HIV. *J. Immunol.* **140**:1139-1141.
 25. Pyren, K. H., H. D. Ochs, M. T. W. Dufford, and R. J. Wedgwood. 1987. Perinatal infection with human immunodeficiency virus: specific antibody response by the neonate. *N. Engl. J. Med.* **317**:611-614.
 26. Reddy, M. M., and M. H. Grieco. 1988. Elevated soluble interleukin-2 receptor levels in serum of human immunodeficiency virus infected populations. *AIDS Res. Hum. Retroviruses* **4**:115-120.
 27. Reddy, M. M., M. Lange, and M. H. Grieco. 1989. Elevated soluble CD8 levels in sera of human immunodeficiency virus infected populations. *J. Clin. Microbiol.* **27**:257-260.
 28. Reddy, M. M., S. J. Sorrell, M. Lange, and M. H. Grieco. 1988. Tumor necrosis factor and HIV P24 antigen levels in serum of HIV infected populations. *J. Acquired Immune Defic. Syndr.* **1**:436-446.
 29. Rifai, N., and A. Morales. 1989. Immunoturbidimetry of β_2 -microglobulin in serum. *Clin. Chem.* **35**:1996-1997.
 30. Rogers, M. F., C.-Y. Ou, M. Rayfield, P. A. Thomas, E. E. Schoenbaum, E. Abrams, K. Krasinski, P. A. Selwyn, J. Moore, A. Kaul, K. T. Grimm, M. Bamji, G. Schochetman, and the New York City Collaborative Study of Maternal HIV Transmission and Montefiore Medical Center HIV Perinatal Transmission Study Group. 1989. Use of the polymerase chain reaction for early detection of the proviral sequences of human immunodeficiency virus in infants born to seropositive mothers. *N. Engl. J. Med.* **320**:1649-1654.
 31. Rogers, M. F., P. A. Thomas, E. T. Starcher, M. C. Noa, T. J. Bush, and H. W. Jaffe. 1987. Acquired immunodeficiency syndrome in children: report of the Centers for Disease Control National Surveillance, 1982 to 1985. *Pediatrics* **79**:1008-1017.
 32. Rubinstein, A., and L. Bernstein. 1986. The epidemiology of pediatric acquired immunodeficiency syndrome. *Clin. Immunol. Immunopathol.* **40**:115-121.
 33. Ulrich, P. P., M. P. Busch, T. El-Beck, J. Shiota, J. Vennari, K. Shriver, and G. V. Vyas. 1988. Assessment of human immunodeficiency virus expression in cocultures of peripheral blood mononuclear cells from healthy seropositive subjects. *J. Med. Virol.* **25**:1-10.
 34. Zolla-Pazner, S., D. William, W. El-Sadr, M. Marmor, and R. Stahl. 1984. Quantitation of β_2 -microglobulin and other immune characteristics in a prospective study of men at risk for acquired immune deficiency syndrome. *J. Am. Med. Assoc.* **251**:2951-2955.