

Immunoglobulin E levels in relationship to HIV-1 disease, route of infection, and vitamin E status

Miguez-Burbano MJ, Shor-Posner G, Fletcher MA, Lu Y, Moreno JN, Carcamo C, Page B, Quesada J, Sauberlich H, Baum MK. Immunoglobulin E levels in relationship to HIV-1 disease, route of infection, and vitamin E status.

Allergy 1995; 50: 157–161. © Munksgaard 1995.

Our recent studies have demonstrated that in early HIV-1 infection, elevation of plasma immunoglobulin E (IgE) levels precedes the decline of CD4 cell count and is influenced by vitamin E status. In order to further investigate the role of IgE elevation in HIV-1 infection, we determined IgE levels in HIV-1-seropositive and -seronegative intravenous drug users (IDUs) ($n = 38$), in relationship to cellular and humoral immune function, liver enzymes, and vitamin E status. To examine the possible impact of the route of HIV-1 infection on IgE levels, comparisons between the cohorts of the HIV-1-seropositive and -seronegative IDUs and homosexual men ($n = 45$) were also conducted. All HIV-1-seropositive participants had significantly higher ($P = 0.003$) IgE levels than the HIV-1-seronegative subjects. The HIV-1-seropositive IDUs, moreover, demonstrated significantly higher ($P = 0.01$) IgE levels than HIV-1-seropositive homosexual men, despite similar CD4 cell counts. Stepwise regression analysis was used to evaluate the possible variables contributing to the IgE variation. HIV-1 status ($P = 0.0009$), intravenous drug use ($P = 0.014$), CD8 cell counts ($P = 0.0001$), plasma level of vitamin E ($P = 0.006$), and alcohol intake ($P = 0.047$) were significant, accounting for 71% of the IgE elevation. These findings suggest that IgE may serve as a sensitive marker to reflect the evolution of HIV-1 disease in individuals from different risk groups.

M. J. Miguez-Burbano¹,
G. Shor-Posner^{1,2}, M. A. Fletcher²,
Y. Lu¹, J. N. Moreno³, C. Carcamo¹,
B. Page², J. Quesada¹, H. Sauberlich³,
M. K. Baum^{1,2}

¹ Departments of Epidemiology and Public Health, and ² Center for Biopsychosocial Study of AIDS, University of Miami School of Medicine, Miami, FL; ³ Division of Experimental Nutrition, Department of Nutrition Sciences, University of Alabama at Birmingham, AL, USA

Key words: HIV/AIDS; IgE; intravenous drug use; nutritional status.

Marianna K. Baum, PhD
Department of Epidemiology and Public Health
University of Miami School of Medicine
PO Box 01609 (R-669)
Miami, FL 33101
USA

Accepted for publication 4 August 1994

Frequent elevation of immunoglobulin E (IgE) in patients with AIDS has been recognized since the beginning of the epidemic (1) and confirmed in later studies (10, 20). HIV-1-infected individuals exhibit a prominent increase of IgE, approximately twice the level of other immunoglobulin classes, IgG, IgA, and IgM (10). Furthermore, this elevation in serum IgE, which is not significantly correlated with the other immunoglobulins (15, 20), appears to increase with severity of infection, suggesting that disturbance in IgE regulation may serve as a sensitive marker to monitor the course and evolution of HIV-1 disease.

Our recent studies conducted in asymptomatic, HIV-1-infected homosexual men (15, 18) indicate that IgE elevation occurs even during the early stages of the HIV disease, prior to the decline in CD4 cell count, and is influenced by plasma vitamin E status (18). These findings could be particularly important

in HIV-1-infected intravenous drug users (IDUs), who appear to experience rapid disease progression (6, 9) and have recently been documented to have altered nutritional status (3). Although IDUs have been included in immunoglobulin studies (10, 20), factors specific to this group have not been adequately investigated. To further characterize the intravenous-drug-using population, the present study was designed to determine IgE levels in relationship to immune function and nutritional status in HIV-1-seropositive and -seronegative drug users. Comparisons between this IDU cohort and HIV-1-seropositive and -seronegative homosexual men were also conducted to examine the possible impact of HIV-1 infection route on IgE levels. Our findings demonstrate that IgE level is profoundly affected by HIV-1 infection, route of transmission, and vitamin E status.

Material and methods

Subjects

Eighty-three subjects (38 IDUs and 45 homosexual men) were selected for this study from larger cohorts of 230 IDUs and 180 homosexual men, being followed longitudinally for HIV-1 infection and cofactors of HIV disease progression. The IDU cohorts were recruited from an IDU street using population in Miami/Dade County, FL, USA, by means of networks of informal social contacts among IDUs. These cohorts, described in detail elsewhere (16), had not taken part in programs for treatment of drug abuse for at least 6 months prior to recruitment. Participants in the homosexual male cohort were recruited from community physician referrals, local AIDS-related centers, and the University of Miami HIV-1 research screening clinics.

In the present investigation, 21 of the IDUs (16 males and five females) were HIV-1 seropositive (ELISA, confirmed by Western blot), and 17 of the IDU participants were HIV-1 seronegative (14 males and three females). The major drugs abused included cocaine and/or heroin, and marijuana. The IDU participants ranged in age from 23 to 60 years; approximately 50% of the subjects had completed high school.

Of the 45 homosexual men, 25 were HIV-1 infected and 20 were HIV-1-seronegative homosexual controls, ranging in age from 20 to 55 years with a minimum education level of 12th grade. Neither the HIV-1-infected IDUs nor the homosexual men were involved in antiviral chemotherapy regimens at the baseline evaluation. The HIV-1-seropositive homosexual men and all but one of the IDUs were asymptomatic, other than generalized lymphadenopathy.

Clinical procedures

All subjects were given a general physical examination and were questioned regarding their medical history, alcohol intake, demographic information, and sexual behavior. Fasting venous blood samples were obtained at 0800 hours from all subjects to minimize diurnal variation. Blood was collected and centrifuged immediately. All samples were aliquoted and stored at -70°C until ready for analysis.

Immunology

The immunoglobulin E levels were assessed with the Microplate Total IgE, a two-site immunoradiometric technique, supplied by Kallestad. Lymphocyte surface markers (CD4 and CD8) were performed by flow cytometry, using two-color direct immunofluorescence with monoclonal antibodies (Coulter Immunology, Miami, FL, USA).

Beta₂-microglobulin was assessed with a Cellfree Beta₂ microglobulin test kit for quantitative determination (T Cell Diagnostic, Inc).

Nutritional status

Overall nutritional status was determined using levels of serum albumin, determined by standard nephelometric technique, with the normal range considered to be 3.5–5.5 g/dl. Plasma vitamin E status was determined by high-performance liquid chromatography (4). The normal range used for vitamin E was 5–15 $\mu\text{g/ml}$, with levels $< 5 \mu\text{g}$ considered biochemically deficient.

Dietary history

All study subjects were questioned with respect to their dietary consumption and alcohol intake using a semiquantitative food frequency questionnaire, an instrument standardized for nutritional epidemiologic studies (19).

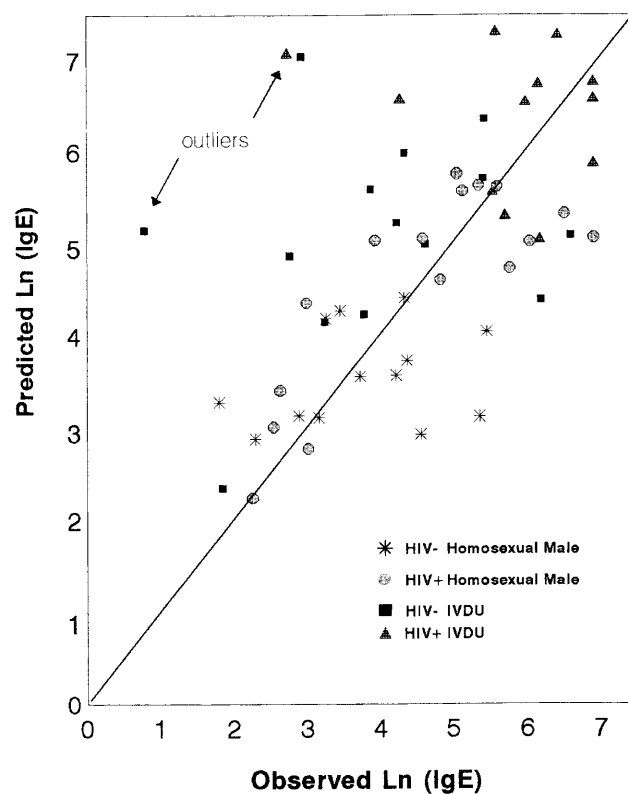


Fig. 1. Scatterplot of predicted and observed log (Ln) of IgE levels is shown. 45° line indicates that there is no systematic pattern of bias, and that regression model predicts observed IgE levels accurately.

Serum markers of liver function

Measurements included serum liver enzyme function (serum glutamic oxaloacetic transaminase (SGOT), and glutamyl transpeptidase (GGTP). Normal levels for the reference laboratory were 0–65 U/l for GGTP, and 0–50 U/l for SGOT.

Statistical analysis

Analyses of the data were performed using SAS statistical packages, following examination of distribution, skewness, and presence of outliers. Transformations were used, when necessary, to correct nonnormal distribution. One-way comparisons were evaluated using Wilcoxon rank sum tests, or Student's *t*-test for independent samples. Correlations of continuous measurements were performed using Spearman procedures, with $P < 0.05$ considered statistically significant for all values.

Multivariate analyses employed ANOVA and ANCOVA. Predictive measurements of HIV status, route of infection, plasma vitamin E, alcohol intake, immunologic variables, and liver enzymes were included in the analysis. Distribution of residuals was examined and outliers were identified. In the final regression model, the outliers were given less weight (10%) than other observations.

Results

Immune function

As indicated in Table 1, the mean CD4 cell count (523.9 ± 283.68) of the HIV-1-seropositive IDUs was similar to that of the seropositive homosexual men (603.5 ± 309.2). There was also no significant

Table 1. Immune function

	IDU		Homosexual	
	HIV-1 (+)	HIV-1 (-)	HIV-1 (+)	HIV-1 (-)
CD4	$523.9 \pm 283.7^{a,a1}$	$864.8 \pm 319^{a,a1}$	603.5 ± 309.2^a	865.4 ± 268.9^a
CD8	848.3 ± 368.7	478.1 ± 181.6	904.7 ± 582.9^a	568.9 ± 163.9
B ₂ M	4.03 ± 2.1^b	2.33 ± 0.81	1.96 ± 0.81^b	1.26 ± 0.42^c

^{a,a1} Significant difference between seropositive HIV-1-infected homosexual men and IDUs, and respective controls at ($P < 0.0001$).

^b Significant difference between seropositive IDUs and homosexual men at $P < 0.0001$.

^c Significant difference between seronegative IDUs and seronegative homosexual men at $P < 0.012$.

difference in CD8 counts between the two groups. Both HIV-1-infected groups, however, had significantly lower CD4 cell counts than their respective seronegative control groups: (864.8 ± 319.0 ; $P < 0.0001$) in seronegative IDUs, and (865.5 ± 268.9 ; $P < 0.0001$) in the seronegative homosexual men.

Significant differences between the groups were noted for immune activation, as determined by level of beta₂-microglobulin. As shown in Table 1, seropositive IDUs exhibited significantly higher levels of beta₂-microglobulin (4.03 ± 2.1) relative to the HIV-1-infected homosexual men (1.95 ± 0.81 ; $P < 0.0001$). Significantly elevated levels of beta₂-microglobulin were also observed in the seronegative IDUs (2.35 ± 0.81), as compared to seronegative homosexual controls (1.26 ± 0.42 ; $P < 0.012$).

Immunoglobulin E levels

A stepwise regression analysis revealed that the level of IgE was significantly associated with HIV-1 status ($P = 0.0009$), CD8 cell counts ($P = 0.0001$), intravenous drug use ($P = 0.014$), plasma level of vitamin E ($P = 0.006$), and alcohol intake ($P = 0.047$). These factors accounted for 71% of the IgE variation. The goodness of the fit is also demonstrated by Fig. 1, which shows the regression line in comparison with the scatterplot of observed IgE levels. No association between SGOT levels and IgE was revealed. A correlation between GGTP and IgE was observed ($P = 0.036$); this factor, however, failed to significantly contribute to the multivariate analysis.

As illustrated in Fig. 2, a pronounced elevation in IgE was noted in the seropositive IDUs, whose IgE levels (444.3 ± 352.4) were significantly higher than the mean IgE level in all other groups ($P = 0.0002$). A direct comparison of the two HIV-1-seropositive groups revealed a significantly higher IgE level in HIV-1-seropositive IDUs (444.3 ± 352.4), as compared to the HIV-1-infected homosexual men (186.4 ± 251.3 ; $P = 0.01$). The HIV-1-seropositive

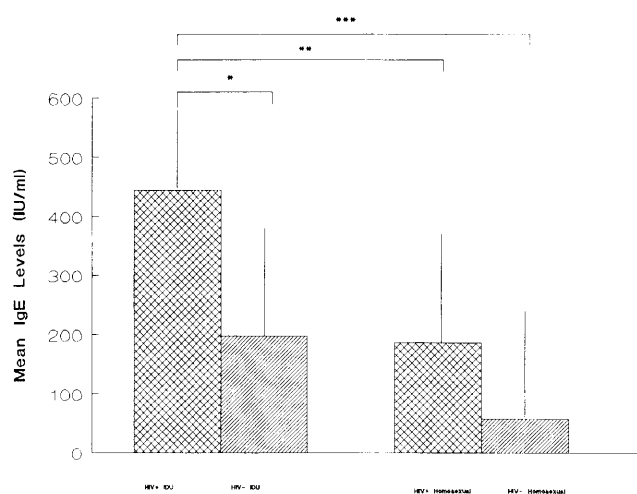


Fig. 2. IgE levels are dramatically elevated in seropositive IDUs as compared to all other groups (** $P = 0.0002$). Direct comparison of seropositive groups revealed significantly higher levels in IDUs, relative to homosexual men, at ** $P = 0.01$ and at $P = 0.02$.

IDUs also showed significantly higher IgE levels than the seronegative IDUs ($P < 0.02$). Both groups of seronegative controls exhibited similar IgE levels.

Nutritional status

General nutritional status, as determined by mean albumin levels, was within the normal range in all groups, although significantly lower levels were observed in IDUs relative to the homosexual men (3.67 ± 0.52 vs 4.14 ± 0.26 ; $P = 0.01$).

The plasma level of vitamin E was significantly lower in the seropositive IDU cohort (5.1 ± 1.7 $\mu\text{g/ml}$), relative to the HIV-1-infected homosexual men, whose mean plasma level of vitamin E was 9.06 ± 6.0 $\mu\text{g/ml}$ ($P = 0.0001$). The plasma vitamin E level in the IDU seronegative controls (5.42 ± 1.89 $\mu\text{g/ml}$) was similar to that of the seropositive IDUs and significantly lower ($P = 0.001$) than that observed in the HIV-1-seronegative homosexual men (10.24 ± 5.11).

Significant differences in dietary vitamin E intake between the groups were observed. Mean vitamin E intake in the HIV-1-infected homosexual group was 306.28 ± 390.5 g, with 84% of the men consuming supplements of vitamin E. The seropositive IDUs, however, consumed 12.5 ± 7.8 g/d of vitamin E, and only 16% of this group consumed supplements. Vitamin E intake in the seronegative homosexual men was 90.58 ± 205.3 g/d, as compared to only 12.2 ± 8.9 g/d in the seronegative IDUs. The proportion of IDUs eating less than the recommended dietary allowance (RDA) of vitamin E was 56%, considerably more than the 16% noted in the homosexual group.

Whereas the mean intake of alcohol and proportion of drinkers (33–36%) was not statistically different among the intravenous drug user and homosexual groups, the amount of alcohol ingested was twofold greater in the seropositive IDUs (26.99 ± 30.85 g/d), as compared to the infected homosexual men (13.2 ± 23.4).

Evidence of hepatic dysfunction, as demonstrated by elevated levels of GGTP (outside the normal range) was shown in both the seropositive IDUs (129.9 ± 112.7 U/l) and the seronegative IDUs (72.0 ± 67.3 U/l). These measurements were significantly higher than those observed in the HIV-1-infected homosexual men (36.44 ± 42.9 U/l; $P = 0.01$) and seronegative controls (18.5 ± 6.9 U/l; $P = 0.01$). Whereas 73% of the seropositive IDUs exhibited elevated GGTP levels, 33% of the seronegative IDUs, and only 12% of the HIV-1-seropositive homosexual men had altered levels of this liver enzyme. The GGTP levels were within the range considered to be normal in the homosexual seronegative men. The mean levels of SGOT were

also significantly higher in the seropositive and seronegative IDUs (44.4 ± 22.8 and 33.1 ± 15.3 U/l), as compared to the seropositive and seronegative homosexual men (29.2 ± 9.0 and 23.7 ± 8.2 U/l) ($P = 0.01$).

Discussion

The results of this study indicate that plasma IgE level is significantly associated not only with HIV status, but with intravenous drug use, immune function, and alcohol intake, as well as with plasma vitamin E level. Whereas IgE levels have been shown to be increased in HIV-1-infected patients (10, 15, 18, 20), our investigation indicates that seropositive drug users exhibit a dramatic and significantly higher IgE level than HIV-1-infected homosexual men, with similar CD4 cell counts. These data indicate that combining various high-risk groups in single cohort studies may not be appropriate.

The prominent IgE elevation demonstrated in HIV-1-infected IDUs was also observed in the HIV-1-seronegative drug users, whose IgE levels were comparable to HIV-1-seropositive homosexual men and significantly higher than the seronegative homosexual men. Both the seropositive and the seronegative IDUs showed increased immune activation, as measured by elevated beta₂-microglobulin levels, providing support for the hypothesis that repetitive antigen challenge of drug abusers may have a profound effect on IgE antibody formation. IgE levels in IDUs may also be increased by hypersensitivity reactions produced by the drugs themselves, or the substances mixed with cocaine and heroin (talcum powder, lidocaine, quinine, sucrose, lactose, etc.), and/or even the bleach used to clean the needles (2, 5, 7, 12, 17). Other factors, including exposure to bacteria and blood-borne viruses other than HIV through needle sharing (6), alcoholism, and liver disease (11), may also influence IgE levels in drug users.

Consistent with previous evidence demonstrating suppressor T-cell regulation of ongoing IgE antibody formation (8), the present investigation revealed a significant relationship between IgE level and CD8 cell counts. This finding supports an earlier suggestion that IgE elevation could be due to a selective defect of suppressive CD8 cells (10). Our data are also in agreement with earlier findings of higher IgE response in HIV-1-seropositive hemophiliacs than in HIV-1-infected homosexual men, supporting the hypothesis that repeated antigenic stimulation may affect the IgE response, depending upon the route of infection (13).

In accord with our previous findings (18), low plasma vitamin E was associated with altered IgE levels. Plasma levels of vitamin E, which were significantly diminished in IDUs as compared to homo-

sexual men, may reflect an increase in oxidative stress due to constant antigenic challenge in IDUs. As a large number (47.3%) of the IDUs consumed vitamin E at levels below the recommended dietary allowance, inadequate plasma vitamin E status may also have resulted from poor dietary intake. In addition, the presence of hepatic disease and/or the additive effects of multidrug abuse and alcohol intake may have contributed both to the low plasma vitamin E and the elevated IgE levels (14).

HIV-1-infected individuals have been documented to exhibit a preferential increase of IgE over other immunoglobulin classes, IgG, IgM, and IgA (1, 10). The increase in serum IgE, which is not significantly correlated with other immunoglobulins (10, 20), occurs even during the early stages of HIV-1 infection (15), and appears to increase with severity of disease. Moreover, this elevation occurs prior to the decline of CD4 cell count (15, 18), suggesting that disturbance in IgE regulation may serve as a sensitive marker to monitor the evolution of HIV-1 disease in individuals from different risk groups. Further investigations will be required to determine the specific mechanisms by which IgE is altered and whether correction can be of therapeutic value in slowing disease progression.

Acknowledgments

We thank K. Pratt and V. Lopez for excellent technical and editorial assistance. This study was supported by NIMH 1-P50-MH42555 (MKB) and Fogarty International Training Grant D43-TW00017-5 (MKB).

References

1. AMMANN AJ, ABRAMS D, CONANT M, et al. Acquired immune dysfunction in homosexual men: immunologic profiles. *Clin Immunol Immunopathol* 1983; 27: 315-25.
2. BARR E, DOUGLAS J, HILL CM. Recurrent acute hypersensitivity to quinine. *BMJ* 1990; 301 (6747): 323.
3. BAUM MK, SHOR-POSNER G, QUESADA J, et al. Nutritional abnormalities in intravenous drug users: impact of HIV-1 status. IX International Conference of AIDS, Berlin, Germany, 1993: 1020.
4. BIERI JG, TERESA J, TOLLIVER BS, CATIGNANI GL. Simultaneous determination of alpha tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. *Am J Clin Nutr* 1979; 32: 2143-9.
5. BONNET MC, DUCAILAR G, DESCHODT J. Anaphylaxis caused by lidocaine. *Ann Fr Anesth Reanim* 1989; 8: 127-9.
6. BRETTLE R. HIV and harm reduction for injection drug users. *AIDS* 1991; 5: 125-36.
7. DAX EM. Drug dependence in the differential diagnosis of allergic respiratory disease. *Ann Allergy* 1990; 64: 261-2.
8. DIAZ-SANCHEZ D, LEE TH, KEMENY DM. Ricin enhances IgE responses by inhibiting a subpopulation of early-activated IgE regulatory CD8 + T cells. *Immunology* 1993; 78: 226-36.
9. GALLI M, JAZZARIN A, SAROCCO A, et al. Clinical and immunological aspects of HIV infection in drug addicts. *Clin Immunol Immunopathol* 1989; 50: S166-76.
10. ISRAEL-BIET D, LABROUSE F, TOURANI JM, et al. Elevation of IgE in HIV infected subjects: a marker of poor prognosis. *J Allergy Clin Immunol* 1992; 89: 68-75.
11. JOSKE RA, TURNER KJ, MURPHY BP. Serum IgE levels in patients with liver disease. *Med J Aust* 1976; 2: 555-7.
12. KAUFMAN AY, KEILA S. Hypersensitivity to sodium hypochlorite. *J Endodont* 1989; 15: 224-6.
13. KHALIFE J, GUY B, CAPRON M, et al. Isotypic restriction of the antibody response to human immunodeficiency virus. *AIDS Res Hum Retroviruses* 1988; 1: 3-8.
14. LEEVY CM, TAMBURRO C, SMITH F. Alcoholism, drug addiction, and nutrition. *Med Clin North Am* 1970; 54: 1567-75.
15. MIGUEZ-BURBANO MJ, MORENO J, GOMEZ O, et al. Possible mechanism of elevated IgE in HIV-1 disease. *FASEB J* 1993; 93 [Abstract 1]: 2901.
16. PAGE BJ, LAI SH, CHITWOOD D, et al. HTLV I/III seropositivity and death from AIDS among HIV-1 seropositive intravenous drug users. *Lancet* 1980; 335: 1439-41.
17. SHAIKH WA. Allergy to heroin. *Allergy* 1990; 45: 555-6.
18. SHOR-POSNER G, MIGUEZ-BURBANO MJ, LU Y, et al. Elevated IgE in relationship to nutritional status and immune parameters in early HIV-1 disease. *J Allergy Clin Immunol* 1995 (in press).
19. WILLETT WC, SAMPSON L, STAMPLER M, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985; 122: 51-65.
20. WRIGHT DN, NELSON RP, LEDFORD DK, et al. Serum IgE and human immunodeficiency virus (HIV) infection. *J Allergy Clin Immunol* 1990; 85: 445-52.