Is there IgA of gut mucosal origin in the serum of HIV1 infected patients?

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Abstract

This study was performed in 77 HIV1 seropositive adult patients to characterise the IgA hyperglobulinaemia seen in the serum during the course of HIV infection. It was shown that both IgA1 and IgA2 subclass concentrations were simultaneously increased but the IgA1 increase was predominant. Secretory IgA (SIgA) concentration was significantly increased and IgA activity to gliadin, bovine serum albumin, and casein could be detected and was correlated with SIgA concentration. In contrast, IgA activity to cytomegalovirus and to tetanus toxoid did not correlate with total IgA concentration. These data suggest the presence of IgA from gut mucosal origin in the serum of these patients. Hyper IgA was inversely correlated with the CD4+ cell number. The increase of all parameters studied varied according to the total IgA concentration in the serum but was also directly related to the stage of immune deficiency in patients with hyper IgA.

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IgA hyperglobulinaemia is a classic immunological abnormality seen in HIV1 disease.¹ In contrast with IgG concentration, which increases very early during the course of HIV infection, hyper IgA is related to immune defect progress and it has been considered to have a prognostic value.² It is usually assumed that hyperglobulinaemia is consecutive to polyclonal stimulation of immunoglobulins production³ by infectious agents especially from the herpes virus group⁴ but also by HIV itself.⁵

Recent data have shown, however, intestinal lesions⁶ with an increase of intestinal mucosal membrane permeability⁷ either secondary to opportunistic germ infection⁸ or occurring earlier during the course of HIV disease directly because of HIV itself.⁹

It was hypothesised that a part of the hyper IgA seen includes IgA from mucosal origin as a consequence of stimulation of the intestinal immune system. The structural characteristics and the activity of IgA to dietary and to non-gut derived antigens were studied and related to the IgA concentration in the serum of 77 HIV seropositive adult patients at different stage of immune deficiency.

Patients and methods

PATIENTS

From a population with sexually or drug transmitted HIV1 infection, we selected 38 patients without immune deficiency (CD4+ cell number higher than 600/mm³) and 39 patients with an immune deficiency (CD4+ cell number lower than 400/mm³, and lower than 200/mm³ in 18 of them). They were classified depending on the IgA concentration in the serum: 32 patients, 28 males and 4 females of mean age 38.5 years (22 to 75) with hyper IgA (IgA concentration ≥ 4.25 g/l) and 45 patients, 35 males and 10 females of mean age 32.6 years (20 to 72) with normal IgA (IgA concentration < 4.25 g/l). One hundred HIV seronegative blood donors from the Centre de Transfusion de Saint-Etienne matched for age and sex were selected as normal controls in each test.

METHODS

Characterisation of IgA in the serum

Enzyme linked immunosorbent assay (ELISA) was used¹⁰ in all studies. Positive and negative controls were systematically included. Normal values defined by mean (2 standard deviations) were calculated from 100 blood donors serum specimens.

Measurement of IgA1 and IgA2 subclass – Serum samples were tested for IgA1 and IgA2 subclass with specific monoclonal antibodies¹¹ using a technique adapted from Conley *et al.*¹² For IgA1 subclass measurement, mouse monoclonal IgG to human IgA1 (Becton Dickinson, Mountain View, CA, USA) were directly coated on polystyrene Microwell immuno-Nunc plates (Nunc, Kamstrup, Roskilde, Denmark) at a concentration of 3 µg/ml in 0.1 M carbonate buffer pH 9.6 overnight at 4°C. For IgA2 subclass measurement, plates were coated with goat polyclonal IgG to mouse γ chain (Cappel Lab, Cochranville, PA, USA) at a concentration of 20 µg/ml in the previous buffer. After washing, mouse monoclonal IgG to human IgA2 (Becton Dickinson) were added at a concentration of 1 µg/ml in 0.05 M phosphate buffer containing 0.05% TWEEN 20 and 0.1% bovine serum albumin pH 7.3, incubated for one hour at 37°C, and then washed three times. Serum samples diluted 1/5000 for IgA1 and IgA2 assays in the same buffer were added in triplicate and incubated

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for one hour at 37°C and then plates were washed three times. Peroxidase conjugated goat polyclonal IgG to human α chain diluted 1/25 000 in the previous buffer were incubated for one hour at 37°C. Enzyme activity was detected by the addition to each well of 100 μ l substrate solution consisting of 4 mM orthophenylene diamine (Sigma, Saint-Louis, USA) in 0.02 M citrate phosphate buffer pH 5 containing 0.04% hydrogen peroxide. After 10 minutes at room temperature, 50 μ l of 1 N HCl were added to stop the reaction. Absorbance of each well was read at 492 nm with a Titertek Multiskan (Flow Lab, Irvine, UK). Serum samples were tested in the presence of serial dilutions of a pool of serum from 100 normal blood donors. The amounts of IgA1 (2 g/l) and of IgA2 (0.2 g/l) in the pool were determined using standard batch n° 5286 kindly donated by Dr J Radl (TNO Institute of Ageing and Vascular Research, Leiden, The Netherlands). Result were expressed as g/l.

Measurement of secretory IgA – Plates were coated with F(ab')₂ fragments of goat polyclonal IgG to human secretory component (kindly provided by Cl Vincent, INSERM U80, Lyon, France) at a concentration of 20 μ g/ml. Serum samples diluted 1/200 were added in triplicate for one hour at 37°C and then incubated with peroxidase conjugated goat IgG to human α chain.¹³ Samples were tested in the presence of serial dilutions of human secretory IgA standard (Cappel). Results were expressed as μ g/ml.

Detection of IgA and IgG activity to dietary antigens

Measurements of IgA and IgG to gliadin – Plates were coated overnight at 4°C with crude gliadin (Sigma), dissolved in ethanol 70%, and diluted at a concentration of 10 μ g/ml in 0.1 M carbonate buffer pH 9.6. Uncoated sites were saturated with a solution of gliadin at 2% in carbonate buffer for one hour at 37°C. Serum samples diluted 1/50 for IgA and 1/100 for IgG in 0.05 M phosphate buffer pH 7.3 containing 1% of rabbit serum (free of gliadin antibody) and 0.05% TWEEN 20 were added in triplicate for one hour at 37°C. After washing, plates were incubated with peroxidase conjugated goat polyclonal IgG to human α chain as previously described or with peroxidase conjugated goat to polyclonal IgG to human γ chain diluted 1/50 000 in the previous buffer.

Measurement of IgA and IgG to BSA – The above procedure was used except that BSA (Sigma) was directly coated after dilution at a concentration of 10 μ g/ml in 0.1 M carbonate buffer pH 9.6. Uncoated sites were saturated with a solution of BSA at 5%. Serum samples were diluted 1/100 for IgA and 1/200 for IgG in phosphate buffer pH 7.3 containing 1% rabbit serum free of antibody to BSA and 0.05% TWEEN 20.

Measurement of IgA and IgG to casein – The above procedure was used except that casein (Sigma) was dissolved in NaOH 0.1 N and

then diluted at a concentration of 10 μ g/ml in 0.1 M carbonate buffer pH 9.6 for coating. Uncoated sites were saturated with a solution of casein at 2%. Serum samples were diluted 1/200 for IgA and 1/400 for IgG in phosphate buffer containing 1% rabbit serum free of antibody to casein and 0.05% TWEEN 20.

In the three previous assays, serum samples were tested in the presence of serial dilutions of a pool containing samples with antibody to gliadin, BSA or casein (positive pool). The optical density for each serum sample was expressed as a percentage of the positive pool at the same dilution from a standard straight line. The pool containing serum samples from normal controls was itself tested as an individual serum in the assay and results were expressed as the ratio between the tested serum and this normal pool.

Detection of IgA and IgG activity to non-dietary antigens

Measurement of IgA and IgG to cytomegalovirus – IgA and IgG activity to cytomegalovirus was determined by a sandwich type ELISA and previously described in detail.¹⁴

Cytomegalovirus specific IgG antibodies were detected by a commercial antibody capture ELISA (Wellcome Laboratories, Dartford, England). Cytomegalovirus specific IgG serology was performed by a sandwich type ELISA: briefly, F(ab')₂ fragments of goat polyclonal IgG to human α heavy chain (Cappel) were used as solid phase capture reagents. After incubation with appropriately diluted samples, the presence of IgA antibodies to cytomegalovirus was shown by addition of peroxidase conjugated purified cytomegalovirus (Wellcome Laboratories, Dartford, England). Serum specimens with absorbance <0.200 at the 1/16 dilution were considered as negative.

Measurement of IgA and IgG to tetanus toxoid – Plates were coated overnight at 4°C with tetanus toxoid (500 Lf/mg) (Institute Pasteur, Paris, France) at a concentration of 10 μ g/ml in 0.1 M carbonate buffer pH 9.6. Uncoated sites were blocked with a solution of gelatin at 2% in carbonate buffer for one hour at 37°C. Serial twofold dilutions of serum specimens in 0.05 M phosphate buffer pH 7.3 containing 0.05 TWEEN 20 and 0.1% BSA were added in triplicate for one hour at 37°C. After washing, plates were incubated with peroxidase conjugated goat polyclonal IgG to human α chain or to human γ chain as previously described.

The titre was taken as the highest serum dilution that gave an optical density twice as great as the background.¹⁵

STATISTICAL ANALYSIS

Results in hyper IgA group were compared with those in the normal IgA group by the Mann-Whitney U test. The relation between total IgA, both IgA subclass concentrations, and IgA activity to dietary antigens with secretory IgA concentration were studied in each patient by the Spearman rank correlation test.

Figure 1: Serum IgA1 and IgA2 subclass and secretory IgA concentration and IgA1/IgA2 ratio depending on total IgA concentration. Mean value of each group is shown by an unbroken line. Dotted line represents the threshold of increase (mean (2 SD)) from 100 normal controls. IgA1, IgA2, SIgA concentration, and IgA1/IgA2 ratio were very significantly increased in hyper IgA patients compared with the normal IgA patient group by Mann-Whitney U test.

Results

MEASUREMENT OF IgA1 AND IgA2 SUBCLASS AND SECRETORY IgA CONCENTRATION IN SERUM

To study the structural characteristics of IgA in patients with hyper IgA in comparison with patients with normal IgA concentration, IgA1 and IgA2 subclass and secretory IgA concentrations were measured in the serum. IgA1 concentration was 7.43 (4.93) g/l in the hyper IgA group and 1.63 (1.11) in the normal IgA group ($p < 0.0001$). IgA2 concentration was 0.35 (0.31) g/l in the hyper IgA group and 0.10 (0.07) in the normal IgA group ($p < 0.0001$). The significant increase of IgA1/IgA2 ratio in the hyper IgA group (40.34 (40.13)) compared with the normal IgA group (18.54 (11.31)) ($p = 0.0219$) showed that the IgA1 concentration increased more significantly than the IgA2 concentration (Fig 1).

Secretory IgA concentration was 6.07 (2.86) $\mu\text{g/ml}$ in the hyper IgA group and 3.25 (1.14) in the normal IgA group ($p < 0.0001$) (Fig 1).

An attempt was made to find a relation between the subclasses of IgA and secretory IgA. A very significant correlation could be shown both with IgA1 ($r = 0.81$; $p < 0.0001$) and IgA2 subclass ($r = 0.50$; $p < 0.0001$) (Fig 2).

MEASUREMENT OF IgA AND IgG ACTIVITY TO DIETARY ANTIGENS

To study the specificity of IgA and IgG to dietary antigens in patients with hyper IgA in comparison with patients with normal IgA concentrations, IgA and IgG activity to gliadin, BSA, and casein were measured in the serum. Anti-gliadin IgA activity index was 6.38 (10.9) in the hyper IgA group and 1.38 (0.73) in the normal IgA group ($p < 0.0001$). Anti-BSA IgA activity index was 1.88 (1.14) in the hyper IgA group and 1.32 (0.6) in the normal IgA group ($p = 0.0016$). Anti-casein IgA activity index was 1.64 (1.87) in the hyper IgA group and 0.98 (0.45) in the normal IgA group ($p = 0.0033$) (Fig 3). IgA activity to the three dietary antigens selected were significantly increased in hyper IgA patients.

An attempt was made to find a relation between the anti-dietary IgA activity and the secretory IgA concentration in the serum. A very significant correlation could be shown between secretory IgA and IgA to gliadin ($r = 0.82$; $p < 0.0001$), IgA to BSA ($r = 0.59$; $p < 0.0001$), and IgA to casein ($r = 0.44$; $p < 0.0001$) (Fig 2).

By contrast, no significant difference was found for IgG activity to the three dietary antigens between the hyper IgA and normal IgA group. Anti-gliadin IgG activity index was 2.12 (1.99) in the hyper IgA group and 1.32 (0.46) in the normal IgA group ($p = 0.097$). Anti-BSA IgG activity index was respectively 2.21 (1.39) and 2.05 (1.53) ($p = 0.4351$). Anti-casein IgG activity index was respectively 1.00 (0.53) and 0.95 (0.72) ($p = 0.2166$).

MEASUREMENT OF PREVIOUS PARAMETERS IN RELATION TO CD4+ CELLS NUMBER

CD4+ cell number in the peripheral blood was expressed in patient groups with normal IgA and hyper IgA.

In the normal IgA group, CD4+ cell number was higher than 600/mm³ in 31 patients and lower than 400/mm³ in 14 patients (six with CD4+ cells <200). In the hyper IgA group, CD4+ cell number was higher than 600/mm³ in seven patients and lower than 400/mm³ in 25 patients (12 with CD4+ cells <200). There was a significant difference in CD4+ cell number between the two groups ($p < 0.0001$) (Table I).

Values of IgA1/IgA2 ratio, secretory IgA, IgA to gliadin, BSA, and casein were not increased whatever the CD4+ cell number when the total IgA concentration was normal. They were increased in patients with total hyper IgA. The increase was higher in patients when the number of CD4+ cells was less than 400/mm³ and higher than 600/mm³ with a significant difference for IgA to BSA ($p = 0.02$), apart from the IgA1/IgA2 ratio, which decreased non-significantly. These results could be only partly explained by the difference in total IgA concentration in hyper IgA patients and a direct relation with immune deficiency stage has to be considered (Table I).

Figure 2: Regression lines of total IgA, both IgA subclass serum concentrations, and IgA activity to dietary antigens on secretory IgA activity. Correlation coefficient and significance are shown.

MEASUREMENT OF IgA AND IgG ACTIVITY TO NON-DIETARY ANTIGENS

In an attempt to examine the specificity to antigens that were not gut derived, IgA and IgG antibodies to cytomegalovirus and to tetanus toxoid were measured in the serum.

IgA antibodies to cytomegalovirus were not detected in any tested subjects. Geometric mean titre of IgG antibodies to cytomegalovirus was 1687 in the hyper IgA group and 2336 in the normal IgA group ($p=0.3906$).

IgA antibodies to tetanus toxoid were detected with titres higher than 10 (from 10 to

40) in five of 45 patients (11.1%) in the normal IgA group and one of 32 patients (3.1%) in the hyper IgA group (Table II). The difference was not significant ($p=0.4751$). IgG antibodies to tetanus toxoid were detected in all subjects with geometric mean titre 1018 in the hyper IgA group and 990 in the normal IgA group ($p=0.7211$).

Discussion

This study, performed in HIV1 infected patients, classified according to the IgA

Figure 3: Serum anti-dietary antigen IgA activity concentration depending on total IgA concentration. Mean value of each group is shown with an unbroken line. Dotted line represents the threshold of increase (mean (2 SD)) from 100 normal controls. IgA to gliadin, to BSA, and to casein activity were very significantly increased in hyper IgA patients compared with the normal IgA patient group by Mann-Whitney U test.

TABLE I Structure and anti-dietary specificity of serum IgA depending on CD4+ cell number

	CD4+ cells (n/mm ³)	Patients (n)	Total IgA (g/l)	IgA1/IgA2 (ratio)	SIgA (μg/ml)	IgA to gliadin (index)	IgA to BSA (index)	IgA to casein (index)
Normal IgA (n=45)	>600	31	2.7 (0.62)	17.35 (9.79)	3.30 (1.21)	1.36 (0.79)	1.26 (0.68)	0.92 (0.47)
	<400	14	3.08 (0.7)	21.38 (14.43)	3.15 (1.00)	1.42 (0.6)	1.44 (0.35)	1.12 (0.39)
Hyper IgA (n=32)	>600	7	6.84 (3.37)	53.48 (40.10)	4.74 (1.33)	2.92 (2.15)	1.29 (0.57)	1.15 (0.77)
	<400	25	7.26 (2.48)	36.51 (40.16)	6.44 (3.07)	7.35 (11.22)	2.04 (1.21)*	1.78 (2.07)

Results are expressed as mean (SD); *p=0.02 by Mann-Whitney U test comparing patients with hyper IgA according to CD4+ cell number.

concentration in the serum, confirms the relation between the increase of serum IgA concentration and the decrease of CD4+ cell number. This is in contrast with the IgG concentration, which increases very early and the IgM concentration, which does not increase during the course of HIV disease (unpublished data).² This discrepancy between circulating immunoglobulins concentrations of different classes suggests that polyclonal activation of B cell may not be the only mechanism responsible for the hyper IgA seen in the serum.³

As it was considered that serum IgA concentration could be related to mucosal immune system stimulation,¹⁶ IgA structural characteristics and anti-dietary antigen IgA activity were studied. By comparison with patients with normal IgA, IgA1 and IgA2 subclass concentrations were both found increased in the serum of hyper IgA patients with a good correlation but the increase of IgA1 was predominant. The highly significant increase of secretory IgA and of IgA antibody activity to gliadin and at a lower activity to BSA and casein correlated with secretory IgA concentration in the serum of hyper IgA patients. This provides strong evidence for the presence of circulating secretory IgA with anti-dietary antigen activity in these patients. All the parameters varied according to the total IgA concentration but were also directly related to the stage of the immune deficiency in patients with hyper IgA. By contrast, IgG activity to dietary antigens was not increased and IgA antibodies to non-gut derived antigens like cytomegalovirus or tetanus toxoid was not detected or was very low whatever the total IgA concentration.

A study analysing subclass distribution, showed that an increase of total serum IgA was accompanied by a preferential increase of IgA1 in connective tissue diseases and to lesser extent in Berger's disease and chronic active liver disease whereas IgA2 percentages tended to be higher in alcoholic cirrhosis and Crohn's disease.¹⁷ Serum concentration of secretory IgA was found increased in lactating women, Crohn's disease, and especially in liver diseases.¹⁸

TABLE II Frequency of IgA antibodies to tetanus toxoid with regard to total IgA concentration

	No of patients (%) with IgA antibodies to tetanus toxoid titres			
	<10	10	20	40
Normal IgA (n=45)	40 (88.9)	1 (2.22)	2 (4.44)	2 (4.44)
Hyper IgA (n=32)	31 (96.9)	0 (0)	0 (0)	1 (3.1)

Results expressed as mean (SD).

High titres of antigliadin IgA antibody in the serum has classically been considered as distinctive of the intolerance to gliadin seen in susceptible subjects with coeliac disease.¹⁹

An increased proportion of polymeric IgA and of secretory IgA to gliadin has also been described²⁰ with a predominance of IgA1 isotype in this disease.²¹

It was shown, however, that serum concentration of IgA antibodies to other dietary antigens are often increased in these patients.²² Moreover high titres of antigliadin IgA antibody and to other dietary antigens were occasionally detected in Crohn's disease, ulcerative colitis, food allergy, and also in Sjögren's syndrome, rheumatoid arthritis and in Berger's disease.²³ Sensitive methods permit even the detection of these antibodies at low titre in normal subjects.²⁴

Enteropathy has been shown to be associated with AIDS.²⁵ Gastrointestinal manifestations are common.²⁶ In some patients, diarrhoea has been shown to result from specific infectious agents⁸ but in others no such course is evident and a direct cytopathogenic effect of HIV itself⁹ or an indirect local immune mechanism²⁷ is suspected. Structural and functional changes, such as low grade bowel atrophy with hyporegeneration, may result from the loss of activated regulatory T cells in the mucosa.²⁸ A reduced number of intestinal IgA containing plasma cells has been reported in AIDS patients.²⁹ Decreased brush border enzyme activities can be found in patients with AIDS³⁰ but also early in the course of HIV infection⁶ causing a malabsorption.³¹

All these data argue in favour of a non-specific immunological hyper-responsiveness against antigens present in the gut lumen probably associated with an increased mucosal permeability resulting from abnormal small intestinal mucosa in HIV infected patients.^{7 32} Respiratory or genitourinary tract mucosa can also participate in the hyper-production of IgA. Impaired clearance of SIgA remains an alternative mechanism.¹⁷ The increased concentration of polymeric or monomeric IgA in the serum of HIV1 infected patients is controversial.^{33 34} The origin of serum secretory IgA antibodies and of IgA to dietary antigens has to be elucidated. They may represent antibodies produced locally in the gut mucosa spilling over into the circulation²⁰ or they may be produced in the bone marrow and in extraintestinal lymphoid tissue as the result of the migration of locally stimulated lymphocytes, or both³⁵ or result from uptake of luminal antigens into the blood.³⁶

This study strongly suggests a disturbance of the gut mucosal immunity increasing during the course of HIV infection as partly responsible for the hyper IgA seen but probably also responsible for other immunological abnormalities and for a defect in the anti-infectious defence mechanisms.

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