

High levels of IgA in HIV-1-perinatally-infected children. Antigen specificity and possible role of increased substance P plasma levels

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The specificity of IgA against food, inhalant, bacterial and fungine antigens as well as for HIV-1 proteins was investigated in 14 HIV-1-infected children (CDC stage P-2) and 15 controls. IgA against food- and inhalant antigens as well as against tetanus toxoid were significantly more often present in the HIV positive children than in controls. No difference between the two groups was present for IgA against *Candida albicans*. A significant increase of substance P, a strong IgA synthesis inducing neuro-peptide, was demonstrated in the plasma of HIV-1 infected children. In conclusion, high levels of IgA seem to reflect a complex immune dysfunction in which many factors are involved. The importance of neuro-immune dysregulation is discussed.

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A B-cell dysfunction in human immunodeficiency virus-type 1 (HIV-1)-infected children is reflected in hypergammaglobulinemia and in particular in high levels of serum IgA (1). The finding of specific anti-HIV-1 antibodies is a useful marker for early diagnosis (2, 3) and prognosis (4) of HIV-1 infection. However, little is known about antibody specificity since only a small portion of serum IgA appears to be directed against HIV-1 proteins.

The mechanisms leading to the high levels of IgA are not clear. An imbalance between T-helper lymphocyte subsets or cytokines has been suggested (5, 6). Moreover, it is already known that neuropeptides and in particular substance P (sP) modulate IgA synthesis (7, 8).

In the present study IgA specificity was evaluated not only against HIV-1 antigens, but also against food, inhalant, bacterial and fungal antigens. The levels of sP was investigated in HIV-1 infected children and the role of this neuropeptide in immune dysfunction discussed.

Subjects and methods

Fourteen consecutive children (6 boys, 8 girls), aged 13 to 73 months (median: 39 months) born to HIV-1-positive mothers were examined at the Cen-

ter for Pediatric HIV-1 infection in Florence, Italy, from October to December 1991. Infection and clinical status were classified according to the Centers for Disease Control Criteria (CDC) (9). All the children were classified as stage P-2 (Table 1). All children were immunised, starting at the age of 3 months with 3 doses of diphtheria toxoid, tetanus toxoid (TT) and inactivated polio virus vaccine. All the patients were treated with intravenous immunoglobulins (Endobulin, Immuno, Pisa, Italy) (400 mg/Kg/months) and with zidovudine (ZDV) (600 mg/m²/day). The age at which the treatments were started is shown in Table 2. The control group was made up of 15 healthy HIV-1 seronegative age- and sex-matched children. Informed consent was obtained from the parents.

Diagnostic assays for viral infection

The infectious status was defined by enzyme immunoassay (ELISA) and Western blot test for HIV-1 antibodies and p24 antigenemia, Polymerase chain reaction for detection of proviral sequences (10, 11, 12) and the isolation of the virus from peripheral blood mononuclear cells (13).

Cytomegalovirus (CMV) infection was identified by virus culture in urine and detection of serum

Table 1. Clinical status, CD4 lymphocyte counts and serum immunoglobulin levels of the HIV-infected children.

Patients	Clinical status*	CD4+ cells/mm ³	CD4/CD8 ratio	IgG g/l	IgA g/l
1	P2A	928	1.40	4460	420
2	P2AD1	20	0.21	1760	491
3	P2AD1	126	1.33	1520	274
4	P2A	1620	2.05	2080	92
5	P2AC	2564	0.44	2850	125
6	P2A	660	0.40	2150	250
7	P2A	1460	0.80	2170	110
8	P2AD2	613	1.11	2090	1110
9	P2AF	1111	1.20	1300	260
10	P2AB	460	0.80	1310	526
11	P2AB	460	1.03	1570	320
12	P2A	640	0.62	2090	182
13	P2A	1435	0.41	3250	107
14	P2AB	259	0.31	1190	444

*based on the Centers for Disease Control classification system.

specific IgM. Antibodies to capsid, nuclear and early antigens of Epstein Barr virus (EBV) were investigated by immunofluorescence.

Assays for specific IgA

In order to improve the sensitivity and specificity of IgA assays, IgG and IgM were removed from serum samples by protein G as previously described (14). ELISA for detection of anti-alpha gliadin IgA specific antibodies was used (alpha-Gliatest IgA-Eurospital, Italy).

IgA antibodies to alpha-lactalbumin (ALA), beta-lactoglobulin (BLG), ovalbumin (OA), *Dermatophagoides pteronyssinus* (DP) or grass mix (GM) were determined by ELISA using the method of Voller et al (15) with some modifications. Briefly, 10 µg/ml purified ALA, BLG or OA (Sigma, St Louis, MO) and 50 µg/ml purified DP or GM were used for the coating of the microtiter plate wells. Alkaline phosphatase-labelled mouse monoclonal antibody to human IgA (Janssen Biochimica, Geel, Belgium) were used for IgA detection. Results were expressed as optical density after background values (obtained with the diluting buffer) were subtracted. We considered positive values those that were at least three times the mean observed in a pool of control sera. IgA antibodies to *Candida albicans* (CA) and TT were determined by an ELISA method (Nuclear Laser Medicine, Milan, Italy).

IgA against HIV-1 antigens were tested by an ELISA (Biochrom HIV-pentELISA, Berlin, Germany). Synthetic peptides of the individual structural proteins of the HIV-1 (p15, p18, p24, gp41, gp120) were used in optimised mixtures for the coating of the wells of microtiter plates.

CD4-CD8 assay for the detection of positive lymphocytes

Monoclonal antibodies were employed to evaluate T-cell subsets with a FACScan cytofluorimeter (Becton Dickinson, Mountain View, CA, USA).

Assay for evaluation of substance P plasma levels

For the evaluation of the levels of sP, plasma were extracted with acidic ethanol, air-dried and reconstituted with assay buffer the day of the test, and then detected by radioimmunoassay (Substance P ¹²⁵I RIA kit. INCSTAR Corporation, Stillwater, MO, USA). The results were expressed as mg/l.

Clinical and laboratory parameters for gastrointestinal functional status

For the evaluation of malabsorption serum levels of iron, ferritin, transferrin, cholesterol, triglycerides, vitamin B12, folate, as well as growth parameters have been considered.

Statistical analysis

The Wilcoxon rank sum test and the Fisher's exact test were used for statistical analysis.

Results

Serum IgA levels were significantly higher in infected children (median 267, range 92–1110 g/l (Table 1) than in controls (median 79, range 61–119 g/l) ($p < 0.01$).

IgA antibodies against at least one of p15, p18, p24, gp41, gp120 antigens were detected in 12 (85%)

Table 2. Age of patients at time of evaluation, age at which therapies were instituted and growth parameters.

Patients	Months			Weight percentile level	Length percentile level
	Age at evaluation	Age at which IVIG therapy was instituted	Age at which ZDV therapy was instituted		
1	13	11	11	50	50
2	72	48	72	20	20
3	73	36	71	50	75
4	28	13	26	90	75
5	42	20	40	10	25
6	28	10	27	25	25
7	52	11	50	50	50
8	29	22	24	10	10
9	17	10	10	25	25
10	16	8	10	25	10
11	36	29	29	10	30
12	68	43	60	75	50
13	70	60	60	25	25
14	49	38	42	50	50

IVIG = Intravenous immunoglobulins.

ZDV = zidovudine.

Table 3. Specific anti-HIV-1 IgA in HIV-1 infected children.

Patients	p15	p18	p24	gp41	gp120
1	-	-	-	+	+
2	-	+	+	+	+
3	+	+	+	+	+
4	-	-	-	-	-
5	-	-	-	+	+
6	+	+	+	+	+
7	-	+	-	+	+
8	+	+	+	+	+
9	-	-	-	-	-
10	+	+	+	+	+
11	-	+	-	+	+
12	-	-	-	-	+
13	-	-	+	+	+
14	-	+	+	+	+

of the 14 infected infants (Table 3). No anti-HIV-1 IgA were detected in 15 samples from healthy seronegative children (control group).

Anti-food-antigen IgA were detected in 12 (85%) of the 14 infected children. The response against each food antigen in HIV-1-infected children and controls is reported in Table 4.

Anti-DP IgA were detected in respectively in 8/14 (57%) of seropositive children and in none of the controls. Anti-GM IgA were detected in 9/14 (64%) infected children and in 0/15 controls (Table 4). Anti-TT and anti-CA IgA were detected respectively in 10/14 (71%) and in 6/14 (42%) patients and in 3/15 (20%) and in 5/15 (33%) controls (Table 4).

No correlation was found between total serum IgA levels and positivity for specific IgA antibodies. However, one of the two children who were negative for specific anti-HIV antibodies had the lowest level of serum IgA (92 g/l) and serum IgA against the other tested antigens were undetectable.

The preparation of immunoglobulin regularly used intravenously in all the patients was tested for specific IgA antibodies against all the antigens studied and no positivity was found. The CD4 positive lymphocyte counts are shown in Table 1.

Substance-P levels were significantly higher in HIV-1-infected children (862 ± 246 mg/l) than in control group (238 ± 102 mg/l) ($p < 0.001$). Correlation between sP levels and total serum IgA levels and correlation between sP levels and CD4/CD8 ratios were not statistically significant.

No difference in serum levels of iron, transferrin, ferritin, vitamin B12 and folate were found between the two groups. Cholesterol and triglycerides tended to be higher, although not reaching statistical significances in HIV-1 infected children than in controls. Growth parameters are shown in Table 2. None of the patients and controls showed gastrointestinal or allergic symptoms.

Discussion

Our results show that IgA antibodies are directed against a variety of antigens in HIV-1 infected children. Specific anti-HIV IgA were not found in two children. In one of them IgA against food and inhalant antigens, as well as against CA and TT were not found. This child had the lowest value of total IgA of all. There was no significant correlation between values of serum IgA and presence of specific IgA against viral and not-viral antigens. Further studies on larger populations will help clarify the relationship between severity of clinical disease and IgA specificity.

The presence of IgA against at least one food antigen in most of the HIV-infected children could possibly suggest a gastrointestinal dysfunction. Intestinal engagement is an important problem in patients with HIV-1 infection (16). A decreased intestinal immunity (17) and opportunistic enteric infections (18), as well as the fact that HIV-1 itself affects the gastroenteric mucosa (19) may induce an intestinal malfunction and a dysregulation in the mucosal immune system and may lead to positivity in anti-food-antigen IgA (20).

Other ubiquitous antigens were also investigated. The majority of HIV-1-infected children had serum IgA antibodies against *Dermatophagoides pteronyssinus*, pollen antigens and TT. These results suggest that a gastrointestinal dysfunction, though a prominent component in HIV-1 disease is not a sufficient cause to explain high serum IgA levels directed against defined antigens.

The presence of anti-tetanus IgA antibodies is remarkable since IgG response against vaccines is usually depressed in HIV-1-positive children. We could not test specific anti-TT IgG since all the children studied routinely receive intravenous immunoglobulins. However, it is difficult to imagine that their IgG response was different from what has been reported by other authors (21).

Table 4. IgA positivity against different antigens in HIV-1 infected children and controls.

Antigen	Patients (= 14)		Controls (= 15)		p
	n	%	n	%	
Gliadin	6	43	0	0	0.0063
Alpha-lactalbumin	5	36	0	0	0.0169
Beta-lactoglobulin	10	71	1	7	0.0004
Ovalbumin	7	50	0	0	0.0022
Casein	8	57	0	0	0.0007
<i>Dermatophagoides pteronyssinus</i>	8	57	0	0	0.0007
Grass mix	9	64	0	0	0.0002
Tetatus toxoid	10	71	3	20	0.0073
<i>Candida albicans</i>	6	43	5	33	ns

The increased IgA levels most likely result from HIV-1-related polyclonal activation of B cells (22), which is characteristic of the immunodeficiency in HIV-1 infection. Both EBV and CMV are polyclonal B cell activators and are believed to contribute to this phenomenon. The neurotropism of HIV-1 has been described (23) and direct damage to the central and peripheral nervous system by the virus might lead to alteration of the neuroimmune axis and to cytokine dysregulation (24). This hypothesis might explain the high sP levels we describe in the present study. Hypergammaglobulinemia and hyper-IgA could both be immunological consequences of this NP imbalance. Actually, sP has been reported to enhance immunoglobulin secretion and, in particular, IgA, both *in vitro* (7) and *in vivo* (9).

Other possible causes of hyper-IgA in HIV-infected children include an imbalance between Th1 and Th2 helper T cells that might influence IgA B cell development and cause the IgA level increase in HIV-1 infected children (25). Further studies will clarify which one of the involved mechanisms is the principal cause of IgA synthesis dysregulation in HIV-1-infected children.

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