Clin Exp Immunol 1997; **109**:47–53

Quantification of IgA and IgG and specificities of antibodies to viral proteins in parotid saliva at different stages of HIV-1 infection

O. CARTRY, P. MOJA, A. QUESNEL, B. POZZETTO, F. R. LUCHT & C. GENIN *Groupe Immunité des Muqueuses et Agents Pathogènes, University of Saint-Etienne, Saint-Etienne, France*

(Accepted for publication 21 March 1997)

SUMMARY

Paired sera and parotid saliva from 75 HIV-1-infected patients, divided in three equal groups with $CD4^+$ cell counts > 500, 200–500 and < 200/mm^3, respectively, were analysed for IgG, IgA and secretory IgA (sIgA) concentrations and for IgG and IgA antibody directed to HIV-1. Twenty-nine age-matched HIV^- subjects were used as controls. In serum the concentrations of immunoglobulins were significantly increased in HIV-infected subjects compared with controls, and a progressive increase of IgA and sIgA was noticed while the $CD4^+$ cell count decreased. In contrast, concentrations of IgA and sIgA were not different in parotid saliva between the four subject groups. By an ELISA test directed towards HIV-1 proteins, 73 of the 75 serum specimens from the HIV-infected subjects (97%) and 43 of the corresponding saliva (57%) were found positive for specific IgA antibodies to HIV-1, with an even distribution among the three groups of patients. By Western blotting multiple specificities of IgA to HIV-1 proteins were not frequently found in patients. By contrast, in spite of an IgG concentration in saliva about 100 times lower than that of IgA, reactivities were significantly higher for IgG than for IgA antibodies, especially to *env* and to *pol* HIV-1 products. Altogether, these data suggest that the regulation of IgA production in HIV-infected subjects is independent in serum and in parotid saliva. This imbalance of IgA/IgG antibodies to HIV-1 at the mucosal level appears to be a specific feature of HIV-1 infection, and may raise important issues in terms of local protection after immunization.

Keywords IgA IgG HIV-1 mucosal immunity parotid saliva

INTRODUCTION

In most cases, transmission of HIV-1 occurs through the mucosal route after heterosexual or homosexual intercourse. HIV-1 has been detected in several mucosal sites [1–4] and in various body fluids [5–8]. Furthermore, opportunistic infections are frequently located to mucosal surfaces in AIDS patients. These data demonstrate that the mucosal immune system is largely involved in the course of HIV-1 infection. However, it is not established whether changes in the gut-associated lymphoid tissue (GALT) during the course of HIV-1 infection parallel changes in the systemic immune system. At the present time, it has been shown that the number of $CD4^+$ lymphocytes in AIDS patients is relatively preserved in the lamina propria of the gut despite the massive depletion in peripheral blood [9].

Most of the antibodies produced in human mucosae are secretory IgA (sIgA). They play an important protective role against locally transmitted viral infections [10]. A decrease of IgA-producing cells in the lamina propria of HIV-infected subjects has been reported [11,12]. IgA and IgG antibodies to HIV-1 have been detected in various body fluids, including parotid saliva, cervicovaginal secretions, semen, breast milk, tears, intestinal fluid and rectal secretions [13–20]. It can be hypothesized that some of these antibodies may have a protective effect, and mucosal immunization trials are in progress.

Parotid saliva is a secretory fluid that can be used as a model for the mucosal humoral immune response in the digestive tract [21]. The aim of this study was to compare the concentrations and the specificities to HIV-1 proteins of IgA and IgG in parotid saliva and in serum, and to evaluate their variations during the course of HIV-1 infection.

Correspondence: Professor C. Genin, Laboratoire d'Immunologie, Faculté de Médecine Jacques Lisfranc, 15 rue A. Paré, 42023 Saint-Etienne Cédex 2, France.

MATERIALS AND METHODS

Subjects

Serum specimens and stimulated parotid saliva samples were collected from 92 adults with sexually or drug-transmitted HIV-1 infection. The $CD4^+$ cell count in peripheral blood was determined by flow cytometry, and patients were classified into three groups: (i) group 1 included 30 patients with a $CD4^+$ cell count > 500/mm^3 (703·3 ± 184·66/mm^3 (mean + s.d.)) (male/female ratio 21/9; mean age 35·7 years; range 23–61 years); (ii) group 2 included 31 patients with a $CD4^+$ cell count between 200 and 500/mm^3

($351{\cdot}64 \pm 100{\cdot}19/mm^3$) (male/female ratio 27/4; mean age 34·5 years; range 18–60 years); (iii) group 3 included 31 patients with a $CD4^+$ cell count < $200/mm^3$ ($98{\cdot}16 \pm 134{\cdot}54/mm^3$) (male/female ratio 25/6; mean age 38·1 years; range 26–58 years). According to the disease stage, the study population consisted of 60 healthy asymptomatic subjects (Centres for Disease Control and Prevention (CDC) stage II), 14 patients with persistent generalized lymphadenopathy (CDC stage III), 18 patients with one or more AIDS-defining illnesses (CDC stage IV). A group of 30 healthy HIV^- volunteers matched for age and sex were used as controls. The protocol was approved by the Comité de Protection des Personnes dans la Recherche Biomédicale Rhône-Alpes Loire, and all subjects gave written informed consent for participating in the study.

Collection of samples

Parotid saliva was collected between 10 am and 1 pm (at least 1 h after a meal) for 10 min with a flexible catheter introduced into the parotid duct (Centracath ORX, Vygon, France). The production of saliva was stimulated by a drop of green lemon applied every 30 s to the lateral face of the tongue. The samples were immediately chilled on ice and stored as aliquots at −80°C until use. The concentration of albumin, measured by a nephelometric method, was used to evaluate the contamination of parotid saliva by blood during the sampling process. Peripheral blood was collected simultaneously to saliva and serum was stored as aliquots at −80°C until use.

ELISA for total IgA and IgG in serum and in parotid saliva

The ELISA technique used for measuring total IgA and IgG was performed as previously described in detail [22]. Concentrations of IgA and IgG were extrapolated from reference curves generated by assaying dilutions of a pool of serum specimens from 100 healthy blood donors whose IgA and IgG concentrations had been determined by immunonephelometric technique. Concentrations were determined as the mean of the results of two two-fold dilutions whose absorbances were in the range of sensitivity of the reference curve linear part.

ELISA for sIgA in serum and in parotid saliva

The assay was conducted as described above, except for the following changes. Plates were coated with $F(ab')_2$ fragments of a goat polyclonal antiserum directed to the human secretory component (kindly provided by C. Vincent, INSERM U80, Lyon, France). The antiserum to human α-chain was the same as above. Concentrations were extrapolated from a standard curve established with dilutions of a purified sIgA standard (Cappel, Cochranville, PA). A pool of 30 parotid saliva specimens from healthy subjects without antibodies to HIV was used as control.

Processing of serum and saliva specimens for IgG removal

For the detection of IgA antibodies to HIV, serum or parotid saliva diluted in 0·05 M PBS containing 0·05% Tween 20 and 0·1% bovine serum albumin (BSA) was systematically mixed to lyophilized sheep antiserum to human IgG (RF-Absorbant; Behringwerke AG, Marburg, Germany), as previously described [22]. After overnight incubation at 4°C, the mixture was centrifugated at 1500 ***g*** for 15 min and the supernatant was collected. As determined in preliminary testing of IgA and IgG concentrations by ELISA, two successive absorption steps were required for parotid saliva samples and three for serum specimens in order to obtain a complete depletion in IgG without impairing the concentration of IgA.

Detection of IgA antibodies to HIV by ELISA

For the screening of IgA antibodies to HIV, a commercial kit containing viral lysates as antigens (Rapid Elavia Mixt; Diagnostics Pasteur, Marne-la-Coquette, France) was used with a modified technique: 100 μl of absorbed serum (final dilution 1:50) or saliva (final dilution 1:2) were added to the wells of a microplate coated by the manufacturer with HIV antigens. The IgA antibodies were revealed by a peroxidase-conjugated goat polyclonal antiserum to human α-chain (Cappel). Absorbances were read spectrophotometrically at 492 nm. Samples with optical densities (OD) higher than the cut-off serum value supplied by the manufacturer were considered to be positive for IgA antibodies to HIV.

Immunoblot analysis of antibodies to HIV-1

In parotid saliva specimens, IgG antibodies to HIV-1 proteins were detected by a commercial immunoblot kit using SDS–PAGE-treated viral lysates as antigens (New Lav Blot; Diagnostics Pasteur), according to the manufacturer's recommendations. The same strips were used for the detection of specific IgA antibodies using IgG-depleted serum and parotid saliva. The strips were soaked in serum (final dilution 1:10) or in parotid saliva (final dilution 1:2) in milk buffer and gently shaken for 1 h at room temperature and overnight at 4°C. After five washing steps, they were incubated in 500 μl of a peroxidase-conjugated goat polyclonal antiserum to human α- or γ-chain (Cappel) diluted 1:100 in milk buffer for 1 h at room temperature under gentle shaking. After washing as above, 2 ml of 0·1 M Tris-saline containing 0·05% (w/v) diaminobenzidine (Sigma, St Louis, MO) and 0·03% (v/v) H_2O_2 were added for a few minutes to develop the colour reaction. The buffers used for sample dilution and for washing were supplied in the kit. The bands were read visually by two independent observers and scored 0 if no band was detected, 1 if the reaction was weak and 2 if the reaction was strong.

Statistical analysis

For the comparison of quantitative results, Student's *t*-test was used. Correlation coefficients were calculated from linear regression analysis. For the comparison of paired qualitative parameters, the χ^2 test was used.

RESULTS

Albumin concentration and blood contamination in parotid saliva

The concentration of albumin in parotid saliva was measured to evaluate the contamination of this fluid by blood. The maximum level of albumin that was considered acceptable in parotid saliva was calculated as the mean of albumin concentration + 2 s.d. in 30 samples of the control group. This value was 10·25 mg/*l*. All samples with a concentration of albumin over the threshold were assessed to be contaminated by blood. According to this criterion, one sample in the control group, five samples in group 1, six samples in group 2, and six samples in group 3 were excluded from further study (Fig. 1).

Concentrations of IgA, sIgA and IgG in parotid saliva and in serum

The comparative results of IgA, sIgA and IgG in serum and in parotid saliva specimens from the three groups of HIV-1-infected

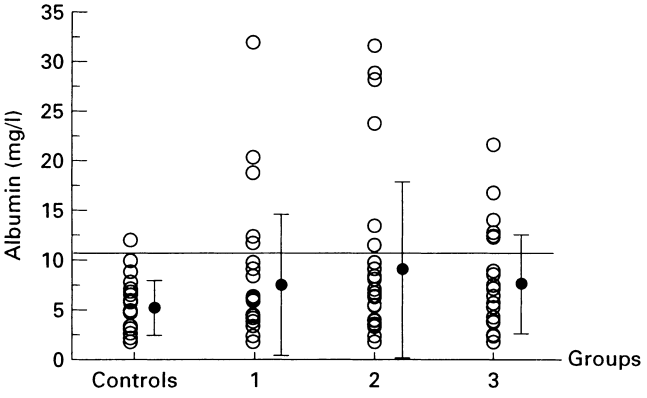

Fig. 1. Concentration of albumin in parotid saliva of controls and HIV-infected subjects with different $CD4^+$ cell counts (the three groups are described in text). The horizontal line corresponds to the level of albumin above which parotid saliva was considered to be contaminated by blood.

subjects and of controls are shown in Table 1. In serum, the concentrations of IgA, sIgA and IgG were significantly higher in HIV-1-infected subjects than in controls. Furthermore, a progressive increase of IgA and of sIgA was noticed in HIV-1-infected subjects while the $CD4^+$ cell count decreased. In contrast, no difference in concentrations of IgA and of sIgA in parotid saliva was found between the four groups of subjects. The concentration of IgG in parotid saliva was higher in HIV-infected subjects than in negative controls, but no change was seen according to the level of immune defect. In addition, a significant correlation was observed between the concentrations of IgA and of sIgA, both in serum specimens and in parotid saliva. Taken together, these results support the finding that in HIV-infected patients the concentration of secretory IgA of mucosal origin increases in serum while the $CD4^+$ cell count decreases, but is stable in parotid saliva.

IgA antibodies to HIV in serum and in parotid saliva

By ELISA, 73 of the 75 serum specimens from the HIV-infected subjects (97%) and 43 of the corresponding parotid saliva (57%) were found positive for specific IgA antibodies to HIV-1. From the 43 parotid saliva samples with HIV-1 antibodies detected by ELISA, only 29 were confirmed positive to at least one viral protein of HIV-1 by Western blotting. The distribution of these 29 positive samples was equal in the three groups of patients (10/25 in group 1, 9/25 in group 2, and 10/25 in group 3).

Specificities of IgG and IgA to HIV-1 proteins by Western blot analysis

The 29 parotid saliva which were found positive for anti-HIV-1 IgA antibodies by Western blotting were also tested for IgG antibodies to HIV-1, and the corresponding serum specimens were tested for IgA antibodies to HIV-1 by the same technique. In serum specimens, anti-HIV-1 IgA was detected against all viral proteins, with 3–10 reactivities per sample (Fig. 2, Table 2). In parotid saliva, anti-HIV-1 IgG was detected to all viral proteins and anti-HIV-1 IgA to all viral proteins but gp41 (Fig. 2, Table 2); 20 of the 29 samples exhibited an IgA reactivity against only one or two proteins. As a whole, reactivity of IgA antibodies was much weaker in parotid saliva than in serum (Fig. 2). Comparisons between the frequency of IgA and IgG antibodies to HIV-1 proteins in parotid saliva revealed that many more subjects had IgG than IgA directed towards the products of *env* (gp160 and gp120) and *pol* (p68, p52 and p34) genes. Conversely, there was no significant difference in the reactivities against the products of the *gag* gene (Table 2). With regard to the $CD4^+$ cell count, a trend to a decrease of IgA to the products of the *pol* and the *gag* gene and of IgG antibodies to the *gag* gene was seen both in serum and in parotid saliva of patients belonging to groups 2 and 3 (Fig. 2). The group sizes were too small to allow conclusive comparisons.

DISCUSSION

First of all, our results confirm the dramatic increase of IgG and IgA levels in serum of patients infected by HIV-1. IgA increase was correlated to the fall in $CD4^+$ cell count, as already reported

Table 1. Concentrations of IgA, sIgA and IgG in serum specimens and in parotid saliva of HIV^- controls (Ctls) and of three groups (G) of HIV^+ subjects according to $CD4^+$ cell count (as described in text)

	Serum			Parotid saliva		
	IgA (g/*l*)	sIgA (mg/*l*)	IgG (g/*l*)	IgA (mg/*l*)	sIgA (mg/*l*)	IgA (mg/*l*)
Controls ($n = 29$)	2·66 ± 1·07	14·78 ± 5·43	18·86 ± 7·43	105·37 ± 73·18	72·83 ± 34·36	0·33 ± 0·18
Group 1 ($n = 25$)	3·48 ± 2·28	15·31 ± 5·75	26·14 ± 8·18	92·52 ± 50·65	90·06 ± 60·18	0·64 ± 0·57
Group 2 ($n = 25$)	4·56 ± 3·15	20·01 ± 8·33	31·07 ± 9·45	123·89 ± 98·47	103·11 ± 63·12	0·74 ± 0·88
Group 3 ($n = 25$)	6·07 ± 3·78	21·39 ± 6·53	26·83 ± 5·91	110·67 ± 70·2	88·31 ± 51·59	0·69 ± 0·76
P values by Student's *t*-test						
Ctls *versus* G.1	NS	NS	0·0009	NS	NS	0·0009
Ctls *versus* G.2	0·01	0·01	0·0001	NS	NS	0·0005
Ctls *versus* G.3	0.0001	0.005	0.0001	NS	NS	0.01
G.1 *versus* G.3	0.0001	0.002	NS	NS	NS	NS

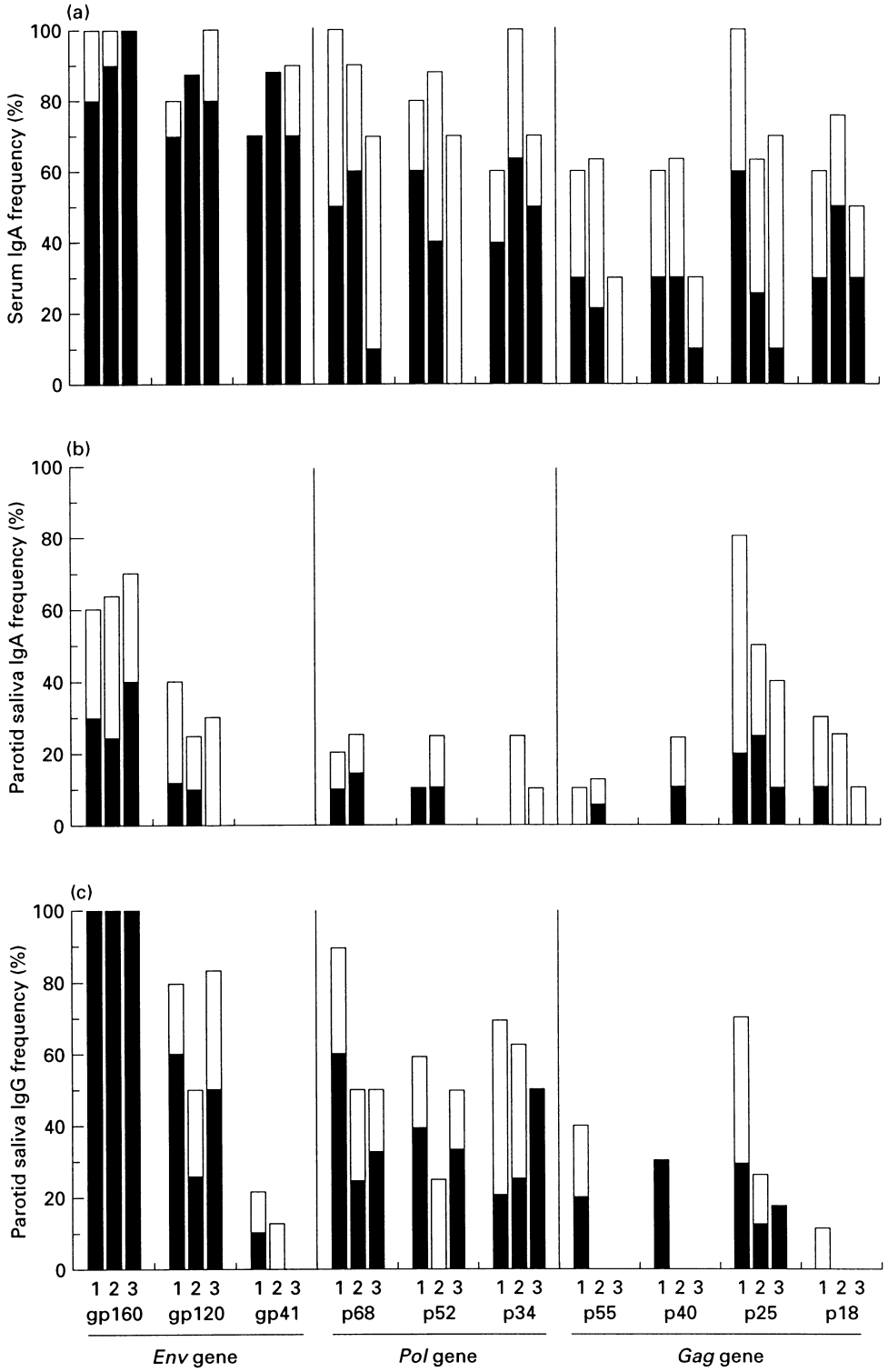

Fig. 2. Repartition of antibodies to HIV-1 proteins by Western blotting in specimens of three groups of HIV-infected subjects with different $CD4^+$ cell counts: the results are expressed as the frequency of patients with low or high reactivity to the various HIV-1 gene products according to the CD4 cell count. (a) IgA in serum. IgA (b) and IgG (c) in parotid saliva. □, Low reactivity; ■, high reactivity. 1, CD4 >500; 2, 200 <CD4 <500; 3, CD4 <200.

[23,24], having been proposed as a surrogate marker to follow the course of HIV infection [25].

In this study, parotid saliva was used to evaluate the variation of IgA and IgG concentrations at the mucosal level during HIV-1 infection. The use of a catheter to collect saliva from the parotid gland guaranteed the purity of our specimens, as demonstrated by the very low concentrations of albumin compared with the results of previous studies using saliva taken from the oral cavity [12,26].

Table 2. Number (%) of HIV^+ subjects with IgA antibodies to HIV proteins in serum and with IgG and/or IgA in parotid saliva by Western blotting ($n = 29$)

		Serum	Parotid saliva		
HIV protein	Gene	IgA antibodies, no. (%)	IgG antibodies, no. (%)	IgA antibodies, no. (%)	*P**
gp160	*env*	29 (100)	29 (100)	19 (65·5)	0·0001
gp120	*env*	25 (86·2)	20 (69·0)	9 (31·0)	0·0001
p68	*pol*	25 (86·2)	20 (69·0)	4 (13·8)	0·0001
p55	*gag*	15 (51·7)	7 (24·1)	2 (6·9)	NS
p52	*pol*	23 (79·3)	14 (48·3)	3 (10·3)	NS
gp41	*env*	24 (82·7)	4 (13·8)	0	NS
p40	*gag*	15 (51·7)	6 (20·7)	2 (6·9)	NS
p34	*pol*	22 (75·9)	18 (62·1)	3 (10·3)	0·0001
p25	*gag*	23 (79·3)	13 (44·8)	18 (62·1)	NS
p18	*gag*	18 (62·1)	4 (13·8)	6 (20·7)	NS

* Statistical comparisons between IgA and IgG in parotid saliva were performed by χ^2 test.

Thus, our results are comparable to mucosal secretions and may exclude any contamination by blood. In contrast with results observed in serum, the concentrations of IgA and sIgA in parotid saliva exhibited no difference between HIV-infected and control subjects. However, the variations of immunoglobulin concentrations were high in parotid saliva, suggesting a rate of secretion depending on patients and/or on a dilution process. In addition, no change was seen with regard to the progression of HIV disease indicated by the decrease of $CD4^+$ cell count. All previous studies performed on whole saliva [12,27,28] and on parotid saliva [29,30] of HIV-infected patients but two [31,32] showed that total IgA concentrations were not increased, and even decreased, suggesting that systemic and mucosal IgA responses were inversely affected in this infection. By contrast with this study, sIgA levels were previously found significantly reduced in AIDS patients compared with asymptomatic HIV-infected subjects and controls [29]. As already noticed [29], this disparity in IgA concentrations between systemic and mucosal compartments may contribute greatly to the reduction of the efficacy of mucosal immunity, leading to frequent infections with opportunistic agents in peripheral sites.

The concentrations of IgG in parotid saliva were remarkably lower than those of IgA, but conversely to IgA, IgG concentrations of our HIV-infected subjects were significantly increased in parotid saliva compared with controls, even before $CD4^+$ cell counts began to decrease in spite of the great variability of IgG concentrations in patients. In HIV infection, a transudation of plasma IgG has been implicated to explain the IgG increase at the mucosal level [19,28,33]. This feature may reflect serious alterations of the mucosal barrier during the course of HIV disease. Although this mechanism cannot be excluded, its importance is probably limited, at least in saliva, since the concentration of albumin—a protein exclusively derived from plasma—was not different in HIV-infected subjects compared with controls. A polyclonal activation of IgG-producing B lymphocytes of mucosal origin could be suggested to explain this IgG increase. As previously reported [34], the finding of such changes at all stages of HIV infection—including the asymptomatic one—confirms that the impairment of mucosal immunity is an early event in the course of HIV disease.

Furthermore, the results of this study suggest that sIgA goes from secretions to the systemic compartment, since the rise of total IgA level was well correlated to that of sIgA, both in saliva and in serum. Thus the dramatic increase of circulating IgA in HIV-infected subjects is probably related to both a polyclonal activation of B cells, producing mainly the monomeric form IgA1 isotype [23], and a crossing of sIgA from mucosal origin [35].

Our study also demonstrated that the frequency of IgA isotype antibodies to HIV-1 structural proteins was lower in parotid saliva than in serum, but did not decrease during the course of HIV-1 infection. Multiple specificities of IgA to HIV-1 gene products could not be detected frequently, especially to gp41 proteins, suggesting a poor mucosal immune response to HIV-1 even at the early stage of infection. In addition, IgA activities to *pol* and *gag* gene products tended to decrease in relation to the $CD4^+$ cell count.

By contrast, IgG reactivities to HIV-1 in parotid saliva were higher and directed to various viral proteins in spite of IgG concentrations about 100 times lower than those of IgA in this fluid. The difference was particularly striking for products of the *env* and of the *pol* genes. Decreasing activity of IgG to *gag* gene products could be noted according to $CD4^+$ cell counts.

In the previous study of IgA to HIV-1 performed in parotid saliva, Archibald *et al.* tested only four samples with an IgA-specific Western blot assay, and three were positive [13]. All other studies were performed in whole saliva, with the risk of blood contamination. IgA antibody activity was tested by ELISA with gp160 only [28,36], or associated with p24 [32] HIV proteins, except in a study of children using Western blot analysis [37]. The salivary anti-HIV activity was also largely carried by the IgG isotype, but the blood [32] or local [28] origin of IgG was controversial. IgG and IgA antibody activity in saliva declined at the symptomatic phase [32].

An IgG predominance in response to HIV has been reported at other mucosal sites, such as seminal fluid [16,38,39], cervico-vaginal fluid [15,40], duodenal fluid [19], breast milk [17,41] and tears [18]. In intestinal fluid, a digestive secretion like parotid saliva, IgG reacted with most HIV-1 antigens, whereas specific IgA were intermittently present and reacted with envelope (gp120 and gp160) and less often with core (p17 and p24) antigens [19], as in this study.

All these data suggest that antibodies of the IgG isotype could play an important role in humoral immunity to HIV at the mucosal level. This feature seems restricted to HIV antigens, since it has been shown that the salivary antibacterial activity of HIV-infected subjects remains located in the IgA isotype [28]. Further studies are needed to determine whether the impaired production of IgA antibodies to HIV antigens is due to an immune tolerance, to a defect in the immunoglobulin heavy chain gene switching, or to a dysregulation of the control of IgA-producing cells by HIV-specific T cells. Interestingly, the proteins involved in this IgA/IgG imbalance are coded by the *env* and the *pol* genes of HIV-1. The products of these genes have also been shown to support most of the protective activity [42–45]. Thus it would be crucial to determine whether the strong IgG activity to HIV at the mucosal level contributes to protection, and which kind of humoral response it would be useful to reinforce by immunization in the perspective of development of mucosal vaccines.

ACKNOWLEDGMENTS

We wish to thank F. Duplat for excellent technical assistance, V. Sommet for help with data collection, and E. Gagnaire for secretarial assistance.

REFERENCES

1 Nelson JA, Wiley CA, Reynolds-Kohler C, Reese CE, Margaretten W, Levy JA. Human immunodeficiency virus detected in bowel epithelium from patients with gastrointestinal symptoms. Lancet 1988; **i**:259–62.

2 Mathijs JM, Hing H, Grierson J, Dwyer DE, Goldschmidt C, Cooper DA, Cunningham AL**.** HIV infection of rectal mucosa. Lancet 1988; **i**:1111.

3 Pomerantz RJ, De La Monte SM, Donegan SP, Rota TR, Vogt MW, Craven DE, Hirsch MS. Human immunodeficiency virus (HIV) infection of the uterine cervix. Ann Intern Med 1988; **108**:321–7.

4 Moyer MP, Huot RI, Ramirez A, Joe S, Meltzer S, Gendelman HE. Infection of human gastrointestinal cells by HIV-1. AIDS Res Hum Retrovir 1990; **6**:1409–15.

5 Ho DD, Schooley RT, Rota TR, Kaplan JC, Flynn T**,** Salahuddin SZ, Gonda MA, Hirsch MS. HTLV-III in the semen and blood of a healthy homosexual man. Science 1984; **226**:451–3.

6 Ho DD, Byington RE, Schooley RT, Flynn T, Rota TR, Hirsch MS. Infrequency of isolation of HTLV-III virus from saliva in AIDS. N Engl J Med 1985; **313**:1606.

7 Vogt MW, Craven DE, Crawford DF, Witt DJ, Byington R, Schooley RT, Hirsch MS. Isolation of HTLV-III/LAV from cervical secretions of women at risk for AIDS. Lancet 1986; **i:**525–7.

8 Thiry L, Spencer-Goldberger S, Jonckheer T, Levt J, Van De Perre P, Henrivaux P, Cogniaux-Leclerc J, Clumeck N. Isolation of AIDS virus from cell-free breast milk of three healthy virus carriers. Lancet 1985; **ii:**891–2.

9 Ullrich R, Zeitz M, Riecken E-O. Enteric immunologic abnormalities in human immunodeficiency virus infection. Semin Liver Dis 1992; **12**:167–74.

10 Ruedl C, Wolf H. Features of oral immunization. Int Arch Allergy Immunol 1995; **108**:334–9.

11 Rodgers VD, Fasset R, Kagnoff MF. Abnormalities in intestinal mucosal T cells in homosexual populations including those with the lymphadenopathy syndrome and acquired immunodeficiency syndrome. Gastroenterol 1986; **90**:552–8.

12 Kotler DP, Scholes JV, Tierney AR. Intestinal plasma cell alterations in the acquired immunodeficiency syndrome. Dig Dis Sci 1987; **32**:129–38.

13 Archibald DW, Barr CE, Torosian JP, McLane MF, Essex M. Secretory IgA antibodies to human immunodeficiency virus in the parotid saliva of patients with AIDS and AIDS-related complex. J Infect Dis 1987; **155**:793–6.

14 Archibald DW, Witt DJ, Craven DE, Vogt MW, Hirsch MS, Essex M. Antibodies to human immunodeficiency virus in cervical secretions from women at risk for AIDS. J Infect Dis 1987; **156**:240–1.

15 Bélec L, Georges AJ, Steenman G, Martin PMV. Antibodies to human immunodeficiency virus in vaginal secretions of heterosexual women. J Infect Dis 1989; **160**:385–91.

16 Bélec L, Georges AJ, Steenman G, Martin PMV. Antibodies to human immunodeficiency virus in the semen of heterosexual men. J Infect Dis 1989; **159**:324–7.

17 Bélec L, Bouquety JC, Georges AJ, Siopathis MR, Martin PMV. Antibodies to human immunodeficiency virus in the breast milk of healthy seropositive women. Pediatrics 1990; **85**:1022–6.

18 Renom G, Bouquety JC, Lanckriet C, Georges AJ, Siopathis MR, Martin PMV. HIV-specific IgA antibodies in tears of children with AIDS or at risk of AIDS. Res Virol 1990; **141**:355–63.

19 Janoff EN, Jackson S, Wahl SM, Thomas K, Peterman JH, Smith PD. Intestinal mucosal immunoglobulins during human immunodeficiency virus type 1 infection. J Infect Dis 1994; **170**:299–307.

20 Mohamed OA, Ashley R, Goldstein A, McElrath J, Dalessio J, Corey L. Detection of rectal antibodies to HIV-1 by a sensitive chemiluminescent western blot immunodetection method. J Acquir Immune Defic Syndr 1994; **7**:375–80.

21 Mestecky J, McGhee JR, Arnold RR, Michalek SM, Prince SJ, Babb JL. Selective induction of an immune response in human external secretions by ingestion of bacterial antigen. J Clin Invest 1978; **61**:731–7.

22 Quesnel A, Pozzetto B, Moja P, Grattard F, Lucht FR, Touraine JL, Gaudin OG, Genin C. Prognostic value of serum immunoglobulin A antibodies to *pol* gene products during HIV-1 infection. Clin Exp Immunol 1993; **91**:237–40.

23 Lane HC, Masur H, Edgar LC, Whalen G, Rook AH, Fauci AS. Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. N Eng J Med 1983; **309**:453–8.

24 Munoz A, Carey V, Saah AJ *et al*. Predictors of decline in CD4 lymphocytes in a cohort of homosexual men with human immunodefiency virus. J Acquir Immune Defic Syndr 1988; **1**:396–404.

25 Davey RT, Lane HC. Laboratory methods in the diagnosis and prognostic staging of infection with human immunodeficiency virus type 1. Rev Infect Dis 1990; **12**:912–30.

26 Marder MZ, Barr CE, Mandel ID. Cytomegalovirus presence and salivary composition in acquired immunodeficiency syndrome. Oral Surg Oral Med Oral Pathol 1985; **60**:372–6.

27 Jackson S. Secretory and serum IgA are inversely altered in AIDS patients. In: McDonald TT, Challacombe SJ, Bland PW, Stokes CR, Heatley RV, Mowat AM, eds. Advances in mucosal immunology. Lancaster: Kluwer Academic Publishers, 1990:665–8.

28 Lu XS, Delfraissy J-F, Grangeot-Keros L, Rannou MT, Pillot J. Rapid and constant detection of HIV antibody response in saliva of HIV-infected patients; selective distribution of anti-HIV activity in the IgG isotype. Res Virol 1994; **145**:369–77.

29 Müller F, Froland SS, Hvatum M, Radl J, Brandtzaeg P. Both IgA subclasses are reduced in parotid saliva from patients with AIDS. Clin Exp Immunol 1991; **83**:203–9.

30 Sweet SP, Rahman D, Challacombe SJ. Serum and saliva immunoglobulin A concentrations show an inverse relationship in HIV infection and AIDS. AIDS 1995; **9**:1288–9.

31 Atkinson JC, Yeh C-K, Oppenheim FG, Bermudez D, Baum BJ, Fox PC. Elevation of salivary antimicrobial proteins following HIV-1 infection. J Acquir Immun Defic Syndr 1990; **3**:41–48.

32 Matsuda S, Oka S, Honda M, Takebe Y, Takemori T. Characteristics of IgA antibodies against HIV-1 in sera and saliva from HIV-seropositive individuals in different clinical stages. Scand J Immunol 1993; **38**:428–34.

33 Sun D, Archibald DW, Furth PA. Variation of secretory antibodies in

parotid saliva to human immunodeficiency virus type 1 with HIV-1 disease stage. AIDS Res Hum Retrovir 1990; **6**:933–41.

34 Lim SG, Condez A, Lee CA, Johnson MA, Elia C, Poulter LW. Loss of mucosal CD4 lymphocytes is an early feature of HIV infection. Clin Exp Immunol 1993; **92**:448–54.

35 Quesnel A, Moja Ph, Lucht F, Touraine JL, Pozzetto B, Genin C. Is there IgA of gut mucosal origin in the serum of HIV1 infected patients? Gut 1994; **35**:803–8.

36 Funkhouser A, Clements ML, Slome S, Clayman B, Viscidi R. Antibodies to recombinant gp160 in mucosal secretions and sera of persons infected with HIV-1 and seronegative vaccine recipients. AIDS Res Hum Retrovir 1993; **7**:627–32.

37 Archibald DW, Johnson JP, Nair P, Alger LS, Hebert CA, Davis E, Hines SE. Detection of salivary immunoglobulin A antibodies to HIV-1 in infants and children. AIDS 1990; **4**:417–20.

38 O'Shea S, Cordery M, Barrett WY, Richman DD, Bradbeer C, Banatvala JE. HIV excretion patterns and specific antibody response in body fluids. J Med Virol 1990; **31**:291–6.

39 Wolff H, Mayer K, Seage G, Politch J, Horsburgh CR, Anderson D. A comparison of HIV-1 antibody classes, titers, and specificities in paired semen and blood samples from HIV-1 seropositive men. J Acquir Immune Defic Syndr 1992; **5**:65–69.

40 Lu XS, Bélec L, Pillot J. Anti-gp160 IgG and IgA antibodies associated with a large increase in total IgG in cervicovaginal secretions from human immunodeficiency virus type 1-infected women. J Infect Dis 1993; **167**:1189–92.

41 Van de Perre P, Simonon A, Hitimana DG *et al*. Infective and anti-infective properties of breast milk from human immunodeficiency virus type 1 infected mothers. Lancet 1993; **341**:914–8.

42 Matthews JJ, Langlois AJ, Robey WG, Chang NT, Gallo RC, Fischinger PG, Bolognesi DP. Restricted neutralization of divergent human T-lymphotropic virus type III isolates by antibodies to the major envelope glycoprotein. Proc Natl Acad Sci USA 1986; **83**:9709–12.

43 Laurence J, Saunders A, Kulkosky J. Characterization and clinical association of antibody inhibitory to HIV reverse transcriptase activity. Science 1987; **235**:1501–4.

44 Berman PW, Gregory TJ, Riddle L. Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160. Nature 1990; **345**:622–5.

45 Neumuller M, Karlsson A, Lennerstrand J *et al.* HIV reverse transcriptase inhibiting antibodies detected by a new technique: relation to p24 and gp41 antibodies, HIV antigenemia and clinical variables. J Med Virol 1991; **34**:55–63.