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Original article

Analysis of IGG and IGG4 in HIV-1 seropositive patients and correlation with biological and genetic markers

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Abstract

We have compared the levels of immunoglobulins G (IgG) and G4 (IgG4) in extreme seropositive patients from the GRIV cohort consisting of 168 patients with slow progression (SP) and 60 with rapid progression (RP) as well as in 173 healthy controls. IgG levels were significantly higher in SP patients than in RP patients ($P = 0.008$), both higher than in seronegative individuals. IgG4 levels were significantly lower in SP patients than in RP patients ($P = 0.001$), both lower than in seronegative individuals. We tried to correlate these levels with biological parameters ($CD4^+$ and $CD8^+$ cells, total lymphocytes, white blood cell counts, percentage of $CD4^+$ cells, and viral load) as well as with genetic markers from Th1/Th2 cytokines (IL2, IL4, IL6, IL10, IL13, and IFNγ). IgG levels were correlated with the percentage of $CD4^+$ cells in SP while IgG4 levels were correlated with $CD8^+$ cell count in SP and with percentage of $CD4^+$ cells in RP patients. Among the parameters measured in SP patients at the time of inclusion in the study, the best predictor of progression towards AIDS was the viral load, the best predictor for stability was $CD4^+$ cell count, but overall, the best predictor for SP evolution (stability vs. progression) appeared to be the percentage of $CD4^+$ cells. Interestingly, correlations between the levels of IgG or IgG4 and the cytokine gene polymorphisms were found, notably in the IL10 gene.
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1. Introduction

During the immune response, the most important function of B cells is to produce high affinity Ag-specific Ig in order to eradicate the infectious pathogens. After an antigenic stimulation, the naïve B cells can undergo isotype switching to produce IgG, IgA, or IgE antibodies. Through the production of various cytokines [1–4], T cells can control the progress of Ig isotype switching by providing signals to the B cells: IL10 promotes the isotype switching to IgG1 and IgG3 [5,6], and IL4 and IL13 to IgG4 and IgE [7–9]. As expected, the production of antibodies is higher in HIV-1 infected patients than in healthy individuals [10]. For example, the level of IgE in AIDS patients was found to be elevated and it has been suggested that this elevated levels of IgE, which are inversely correlated with $CD4^+$ T lymphocytes, could be a negative prognostic factor for rapid progression to AIDS [11–14]. However, the levels of IgG4 were lower in AIDS patients than in healthy population [15]. It has been demonstrated in vitro a competition between IgG4 and IgE antibodies at the antigen-presenting cell level [16]. Therefore IgG4 might play a protective role in Th2-mediated inflammation. In the present study, we have measured IgG and IgG4 levels in the serum of seropositive patients with extreme patterns of progression to

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clarify how their levels could influence disease onset and progression. To perform this study, we employed the GRIV cohort consisting of two sub-populations of French HIV-1$^+$ individuals with extreme phenotypes: slow progression (SP) patients and rapid progression (RP) patients [17–20]. Then, we correlated these immunoglobulin levels with several biological parameters (CD4$^+$ and CD8$^+$ cells, lymphocytes and white blood cells counts, and viral load) related to HIV-1 infection. Since the follow-up after their inclusion was available for some SP patients, we tried to identify which of these parameters (including IgG or IgG4 levels) were predictive markers for disease progression or for stability.

One of the characteristics consistently observed in AIDS patients is the switch of the cytokine production profile from a Th1 to a Th2 type [21]. As a consequence, an alteration of Th1/Th2 balance can influence the isotype switching, which can explain the varying levels of immunoglobulin in HIV$^+$ patients. Finally, we used the data from a previous systematic genetic study of IL2, IL4, IL6, IL10, IL13, and IFNγ cytokine genes [22], performed on the GRIV cohort, to evaluate the correlation between the cytokine gene polymorphisms with IgG and IgG4 production.

2. Material and methods

2.1. Subjects

The GRIV cohort was established in 1995 in France to generate a large collection of DNA samples for genetic studies of candidate human polymorphisms associated with RP and SP to AIDS [18–20,22]. To avoid confounding effects associated with ethnic differences in the genetic analysis, only Caucasians of European descent were recruited from hospital-based AIDS units throughout France. SP patients were defined as asymptomatic individuals who had tested seropositive for HIV-1 for more than 8 years with a CD4$^+$ cell count still above 500/mm^3 in the absence of antiretroviral therapy. A seropositive test result older than 8 years was necessary for inclusion in the study. RP patients were defined by CD4$^+$ count below 300/mm^3 in a period of time of less than 3 years after the last seronegative testing.

Blood from about 300 SP and 100 RP patients has been collected, and serum was prepared and frozen using standard procedures.

The project was approved by the Institutional Review Board of Saint-Louis Hospital (Paris, France) and all patients had to sign an informed consent to participate in the study.

Seronegative French subjects were used as controls (CTR) for the measure of IgG and IgG4 levels.

2.2. Biological parameters and Th1/Th2 cytokines genotyping

The hematologic parameters (CD4$^+$ and CD8$^+$ cell count, percentage of CD4$^+$ cells, total lymphocytes count, and white blood cells count) were obtained from various hospitals at the time of inclusion of the patients. The viral load was measured in the serum taken from the patients at inclusion in the study, according to a standard RT-PCR assay [23].

The genotyping of Th1/Th2 cytokine genes was performed by PCR/sequencing as previously described [22].

2.3. IgG and IgG4 serology

IgG and IgG4 levels were measured in the serum from patients at inclusion in the study. Serum IgG concentrations were determined by immuno-nephelemetry (Image Immunochemistry System, Beckman array system, CA, USA) in 160 SP and 60 RP patients. Serum IgG4 concentrations were measured by a competitive ELISA protocol as previously described [24].

2.4. Statistics

To compare two groups of patients for a given parameter we used a Student's *t* test. For the discriminative analysis in Table 5, we used a Fisher's exact test.

All SNPs found were biallelic with two possible alleles 0 and 1. To compare the genotypes of a SNP between different groups of patients we used a Fisher's exact test comparing the distribution of genotypes (00, 01, 11) or comparing the frequency of each allele or their distribution in a dominant or recessive model. In this work we did not perform any correction for multiple testing.

Two modes have been used for the correlation calculations: first, using data from all patients (as in Table 3), and second, using the "quartiles" method (as in Table 4). The low and high quartiles are, respectively, the 25% lowest and highest data for each parameter. The "quartiles" calculations method only takes in account these extreme data.

3. Results

3.1. Comparison between SPs, RPs, and CTRs

Table 1 presents the serum IgG and IgG4 concentrations and their ratios in the SP, RP and CTR populations, with also a distinction between men and women. The level of IgG was higher among the SP subjects compared to the RP population (P = 0.008), and both higher than the controls (respective means of 22.3 g/l and 19.3 g/l vs. a mean of 10 g/l for controls). Seventy-five percent of the subjects were above the usual threshold of 15 g/l of IgG in the RP and SP groups and there was no notable difference between men and women. The level of IgG4 was decreased with a mean of 0.18 g/l in SP patients and 0.25 g/l in RP patients (P = 0.001) vs. a mean of 0.39 g/l in controls. There were also variations of IgG4 levels according to gender: we found, respectively, a mean of 0.21 and 0.28 g/l in SP and RP men vs. 0.46 g/l in control men and 0.13 g/l for both SP and RP women vs. 0.29 g/l in

Table 1
IgG and IgG4 concentrations. IgG and IgG4 concentrations in SP, RP and CTR groups. The data for CTR subjects were obtained from Aucouturier et al. [24]

		Number of patients	IgG				IgG4			
			Mean	Median	SD	Range	Mean	Median	SD	Range
SP	Global	168	22.35	22.00	7.45	10–51	0.18	0.13	0.21	0.003–1.60
	Male	120	21.85	21.50	6.77	11–48	0.21	0.14	0.25	0.003–1.60
	Female	48	22.89	23.00	8.40	10–53	0.13	0.12	0.08	0.013–0.33
RP	Global	60	19.12	19.00	5.52	8–44	0.25	0.15	0.28	0.01–1.15
	Male	50	19.10	19.00	5.94	8–44	0.28	0.16	0.30	0.01–1.15
	Female	10	20.00	20.00	3.77	16–27	0.13	0.10	0.11	0.02–0.33
CTR	Global	173	9.32	9.32	NA	6.4–12.3	0.39	0.28	0.38	<0.01–2.10
	Male	98	9.25	9.25	NA	6.4–12.1	0.46	0.37	0.37	<0.01–1.87
	Female	75	9.39	9.39	NA	6.5–12.3	0.30	0.18	0.41	<0.01–2.10

control women. These results agree with a previous study performed on seropositive Caucasian patients [15].

We also had access to some biological parameters for the SP and RP patients, such as the $CD4^+$, $CD8^+$, total lymphocytes, and white blood cell counts, as well as their viral load. Table 2 presents the values of the parameters in the SP and RP groups. Clearly, there are differences between SP and RP patients, and the bigger decrease in $CD4^+$ cells compared to the other cell types is clearly noticed through the important decrease of the percentage of $CD4^+$ cells (%$CD4^+$).

3.2. Correlations between the biological parameters

We computed the correlation coefficients between each set of parameters including IgG and IgG4 in the RP and SP groups (Tables 3A and 3B, respectively). We found no significant correlation involving IgG and IgG4 with cell counts or viral load. Significant correlations were obtained between cell counts in both SP and RP groups, such as the one between the $CD8^+$ cell count and the lymphocyte count. The white blood cell counts are well correlated with the lymphocyte counts in SP patients and but not in RP patients: this might be due to the state of inflammation in the RP patients leading to a variable number of leucocytes.

In order to refine our correlation analysis, for each given parameter we also compared the levels of the other parameters (mean, median, SD) among the patients present in the extreme quartiles for that parameter. Table 4A,B summarize the relationships between each couple of parameters in SP and RP patients, respectively: in each point of the table, the values (mean, median, SD) of the parameter marking the column are compared for the patients in the extreme quartiles of the parameter marking the line. For example, the first line corresponds to the comparison for each parameter of the lowest and highest IgG quartiles. In both tables, most parameters in the matrix have a reciprocal correlation, but some asymmetries exist. Thus, if the SP patients for the extreme quartiles of IgG levels present differences in $CD4^+$ cell counts, the extreme quartiles for $CD4^+$ cell counts do not exhibit a significant difference regarding their levels of IgGs (Table 4A). Similarly, if the extreme IgG4 levels seem to present some dependence on the $CD8^+$ cell counts, the extreme $CD8^+$ cell counts do not have a significant dependence on the IgG4 levels. Finally, the extreme years for the date of infection do not seem to influence $CD8^+$ or IgG levels, but the opposite is not true: the extreme levels of $CD8^+$ and of IgG, among the subjects enrolled as SP patients at inclusion, present some dependence on the year of infection (Table 4A).

Since the IgG4 level seems to be very much gender-dependent (Table 1), we established a distinction between male and female patients. There was no difference anymore between men with the highest and lowest IgG4 levels, while a strong difference in haematological cell counts appeared by comparing women with the highest and lowest IgG4 levels. Women with lower IgG4 had much higher $CD4^+$ cell

Table 2
Main biological parameters in SP and RP groups at inclusion. This information was missing for some patients whose Ig levels were measured

		Mean	Median	SD	Range
SP (*N* = 128)	**CD4+**	806.00	728.00	302.00	480–2298
	%CD4+	34.36	33.00	9.87	14–65.20
	CD8+	1096.00	970.00	494.00	264–3011
	Lympho	2401.00	2208.00	752.00	1150–5028
	WBC	6550.00	6390.00	1991.00	3200–16 000
	VL	2.35	2.21	1.18	0–4.96
RP (*N* = 54)	**CD4+**	208.00	176.00	163.00	4–760
	%CD4+	14.31	13.00	8.33	0.60–34
	CD8+	762.00	744.00	418.00	139–2240
	Lympho	1352.00	1311.00	684.00	80–4000
	WBC	4468.00	4200.00	1996.00	1570–11 900
	VL	3.09	3.14	1.13	0–4.89

* Lympho stands for the total lymphocytes count, WBC for the white blood cells count, and VL for viral load given in log.

Table 3
Correlation coefficients A for SP patients B for RP patients

	IgG	IgG4	Ratio Ig	CD4	%CD4	CD8	Lympho	WBC	VL	Year Infect.
(A) *Correlation coefficients for slow progressors*										
IgG	1									
IgG4	0.13	1								
Ratio Ig	–0.16	**0.92**	1							
CD4	–0.23	–0.05	0.02	1						
%CD4	–0.33	–0.08	0.04	0.40	1					
CD8	0.25	–0.01	–0.12	0.23	**–0.66**	1				
Lympho	0.07	0.03	0	**0.53**	–0.43	**0.85**	1			
WBC	–0.03	–0.03	–0.02	0.33	–0.22	0.48	**0.54**	1		
VL	0.18	0.10	0.01	–0.22	–0.22	0.15	–0.01	0.02	1	
Year of Infect.	–0.14	0.11	0.18	0.02	0.03	–0.27	0	0	–0.09	1
(B) *Correlation coefficients for rapid progressors*										
IgG	1									
IgG4	0.14	1								
Ratio Ig	–0.14	**0.95**	1							
CD4	0.04	–0.12	–0.10	1						
%CD4	–0.02	–0.25	–0.24	**0.62**	1					
CD8	0.12	–0.02	–0.01	**0.50**	0.04	1				
Lympho	0.07	0	0.03	**0.63**	0.17	**0.91**	1			
WBC	–0.07	–0.11	–0.05	0.34	0.29	0.47	0.27	1		
VL	0	0.04	0.04	–0.21	–0.25	–0.20	–0.19	–0.14	1	

The significant correlations (>0.5) are marked in bold. Ratio Ig stands for the ratio IgG4/IgG, lympho for the total lymphocytes count, WBC for the white blood cells count and VL for viral load.

counts, higher $CD8^+$ cell counts, and lower viral load. Unlike IgG4s, the differences observed for extreme IgGs regarding $CD4^+$ and $CD8^+$ cell counts and $\%CD4^+$ persisted both in men and in women (data not shown).

Regarding RP patients, there were also some asymmetry points involving the viral load (Table 4B). Since patients were often under treatment with Highly Active Antiretroviral Therapy (HAART), it was obvious to see the absence of reciprocal correlation of extreme $CD4^+$ cell values (often low in RP patients) with the viral load reduced to small values in the treated patients.

As seen on Table 4A,B, most correlations can be found simultaneously in SP and RP populations. However, in RP patients the IgG level did not correlate with the cell counts and viral load, the IgG4 level no longer has a correlation with $CD8^+$ cell counts but rather with the $\%CD4^+$ cells ratio. The $CD4^+$ cell count presents some correlation with $CD8^+$ cell count, the percentage of $CD4^+$ cells has no longer a correlation with $CD8^+$ cell counts and lymphocytes no longer have a correlation with the $\%CD4^+$. To explain this variation between SP and RP patients for IgGs, one can suggest that the cell counts of RP patients have been so disturbed by HIV-1 infection that no correlation can be found since the IgG levels remain rather high (mean 19 g/l in RP patients vs. 22 g/l in SP patients, see Table 1) while the cell counts in AIDS patients have become very disturbed (Table 2). There is no obvious explanation for the correlation of IgG4 with $CD8^+$ counts in SP patients (low IgG4 with higher $CD8^+$) and $\%CD4^+$ in RP patients (low IgG4 with higher $\%CD4^+$). One could think that in SP patients, there is still an excess of $CD4^+$ cells (the inclusion criteria require a $CD4^+$ cell count above $500/mm^3$) and thus the parameter $\%CD4^+$ is less sensitive to the variations of the other cell types: the direct variations of $CD8^+$ cell counts, more representative of a state of activation, might thus more easily correlate to IgG4 production. RP patients are already in a global state of activation; the $CD8^+$ cell count number is correlated with the $CD4^+$ cell count (Table 3B and Table 4B); the parameter IgG4 levels could be more accurately correlated to the balance of $CD4^+$ and $CD8^+$ i.e., % of $CD4^+$ cells. Indeed, RP subjects with low IgG4 have higher $\%CD4^+$ (means of 17.4% vs. 11.4%) but also have higher numbers of $CD4^+$ cells (means of 265 vs. $169/mm^3$) and rather similar levels of $CD8^+$ cells (means of 820 vs. $780/mm^3$). In RP patients it is not such a surprise to see a positive correlation between $CD4^+$ and $CD8^+$ lymphocytes: the patients with higher $CD4^+$ cell counts have a better immune system and can still generate a $CD8^+$ cellular response against the infection (means 920 vs. $460/mm^3$). Such a correlation among SP patients does not exist since globally patients are not crippled by the infection and are not under hyperactivation state.

3.3. Patient follow-up and predictive power of the parameters

The follow-up was available for some patients up to 3 years after inclusion in the study. During this follow-up, some of these patients, termed unstable slow progression (USP) patients, exhibited a decrease of their $CD4^+$ cell count below $400\ CD4^+/mm^3$, or received an antiretroviral therapy because of an increase in their viral load. On the opposite, the stable slow progression (SSP) patients maintained a $CD4^+$ cell count

Table 4
Significant correlations between the extreme quartiles of a parameter with other the parameters A in SP patients, B in RP patients

		IgG	IgG4	Ratio Ig	CD4	%CD4	CD8	Lympho	WBC	VL	Year of Infect.
(A) *Comparison among SPs*											
IgG	14.2 ± 2			+	–	– –	+			++	-
	31.7 ± 7.2										
IgG4	0.03 ± 0.01			+++			–				
	0.4 ± 0.3										
Ratio Ig	0.001 ± 0.001	–	+++								
	0.02 ± 0.01										
CD4	0.53 ± 0.04	**?**				+++		+++	++	–	
	1.19 ± 0.32										
%CD4	23 ± 3.4	– –			++		– –	– –		–	
	47 ± 6.5										
CD8	0.59 ± 0.11	++	**?**			– – –		+++	+		+
	1.75 ± 0.41										
Lympho	1.6 ± 0.21				+++	– –	+++		++		
	3.4 ± 0.61										
WBC	4.57 ± 0.62				++		++	++			
	9 ± 2.1										
VL	0.89 ± 0.72	+			–	–					
	3.87 ± 0.53										
Year of Infect.	1983 ± 0.8	**?**					**?**				
	1989 ± 1.6										
(B) *Comparisons among RPs*											
IgG	14 ± 2.4										
	24.8 ± 5										
IgG4	0.04 ± 0.03			+++		–					
	0.56 ± 0.28										
Ratio Ig	0.002 ± 0.001		+++			–					
	0.03 ± 0.019										
CD4	0.055 ± 0.039					+++	++	++	+	**?**	
	0.39 ± 0.14										
%CD4	5.8 ± 3.5		–	–	+++					**?**	
	23.7 ± 5										
CD8	0.34 ± 0.10				++			+++	+		
	1.21 ± 0.33										
Lympho	0.66 ± 0.24				++		+++		++		
	2.06 ± 0.56										
WBC	2.58 ± 0.6				++		++	++			
	6.57 ± 1.78										
VL	1.85 ± 0.73				–	–					
	4.29 ± 0.4										

?, Asymmetry.
For each parameter marking a line, the patients corresponding to the extreme quartiles were identified (the mean ± SD for these two extreme quartiles are given next to the parameter). For these two groups of patients, the distribution of the values for the parameters in column are then compared by a Student's *t* test in order to see if they were also significantly different. When there is a positive correlation it is indicated as +, when there is an inverse correlation it is marked as –. The number of "+" in the matrix represents the significance of the correlation: 0.01< P <0.05 for "+", 0.001< P <0.01 for "++", and P < 0.001 for "+++" (idem for "–").

less than 15% inferior to their inclusion level. Out of 168 SP patients, we had overall 23 USP and 22 SSP patients. We evaluated the number of SSP and USP patients in each extreme quartile for the parameters tested previously. In Table 5, the best predictor for progression to AIDS is viral load (three USP patients among low VL vs. 11 USP patients among high VL, P = 0.03), the best predictor for stability is $CD4^+$ cell count (two SSP patients among low $CD4^+$ vs. 10 SSP patients among high $CD4^+$, P = 0.01), but the best predictor for patient evolution (stability vs. progression to AIDS) is $\%CD4^+$ (P = 0.04). It may be interesting to notice that too high levels of IgG also appear to be associated with future progression to AIDS (three USP patients among low IgG vs. eight USP patients among high IgG).

Table 5
Predictive value of the parameters among SP patients

		SSP (n = 22)	USP (n = 23)	p1 stability	p2 progression	p3 stability vs. progression
IgG	Low	7	3	0.75	0.19	0.21
	High	5	8			
IgG4	Low	8	6	1.00	0.73	1.00
	High	7	4			
Ratio Ig	Low	5	8	1.00	0.19	0.38
	High	6	3			
CD4	Low	2	5	**0.01**	1.00	0.15
	High	10	4			
%CD4	Low	3	9	**0.06**	**0.21**	**0.04**
	High	10	4			
CD8	Low	8	2	0.33	0.42	0.16
	High	4	5			
Lympho	Low	7	2	1.00	0.42	0.64
	High	7	5			
WBC	Low	6	2	1.00	0.15	0.39
	High	7	6			
VL	Low	2	3	0.43	**0.03**	1.00
	High	5	11			
Year of Infect.	Low	6	5	0.10	0.42	0.59
	High	1	2			

The follow-up during 3 years after inclusion could be clearly confirmed as progression to AIDS (major CD4 cell decrease or therapy given to the patient) for 23 SP patients, thus called USP and stable (a decrease of less than 15% compared to the baseline CD4 level at inclusion) for 22 SP patients, thus called SSP. We took the extreme quartiles for each parameter and computed the number of SSP and USP patients in each quartile. The p1 compares the distribution of SSP patients between the low and the High quartile, p2 the distribution of the USP patients between the Low and High quartiles, and p3 compares the global difference of distribution of SSP and USP patients between the two extreme quartiles.

3.4. Correlations with the genotypes

As shown in Table 1 inter-individual differences in IgG4 levels can be very high. The genetic background of each patient could explain this. IgG4 production is dependent upon the presence of cytokines, in particular IL4 and IL10 [7,25]. Since genotyping data for IL2, IL4, IL6, IL10, IL13, and IFNγ were available from a previous study [22], we tested whether these genetic polymorphisms could be associated with the levels of IgG/IgG4 in the SP and RP populations. We used an approach similar to the one we used to compare the biological parameters: we picked patients with extreme values for IgG, IgG4 and their ratio and checked if there was a significantly different distribution of genotypes between the extreme n-tiles (for n = 2, 3 and 4). We analysed the results in two ways: (i) assuming that SP/RP status of the patients does not influence the IgG or IgG4 production, we pooled the two groups (ii) considering that the immune status of SP or RP patients influences the levels of IgG or IgG4, we separated the two groups. With the first approach, we found some associations with some polymorphisms in IL10 and IL6 genes for both IgG and IgG4 levels (Table 6). With the second one, we found associations with IL10 and IL2 SNPs in SP patients and associations with IL6, IL10, and IFNγ SNPs in RP patients. As they appear significant in all the calculation modes, the SNPs IL10_44, IL10_66, and IL10_302 seem to have stronger impact on IgG and IgG4 levels.

4. Discussion-conclusion

We have undertaken an exhaustive study on the correlations between the IgG and IgG4 levels and biological parameters ($CD4^+$ and $CD8^+$ cell counts, total lymphocytes and white blood cell counts, percentage of $CD4^+$ cells, ratio IgG4/IgG) and genetic markers in the GRIV cohort. We found that the IgG levels were significantly higher (P = 0.008) in SP patients than in RP patients and that the IgG4 levels were significantly higher (P = 0.001) in RP patients than in SP patients. As expected in the context of chronic HIV-1 infection, both groups have an increased mean IgG level compared to controls. On the opposite, the mean IgG4 level was higher in controls than in RP and SP patients. The humoral response to HIV-1 infection is oriented towards the production of IgG1 and IgG3 instead of IgG4. More so, the immune perturbation induced by HIV-1 infection might not allow a normal immune response development leading to production of IgG4.

The difference observed for IgG and IgG4 levels between SP patients and RP patients might be associated to the longer duration of infection in SP patients, to the difference in the immune activation between RPs and SPs (the state of immune activation in RP patients might induce isotype switches enhancing IgG4 production), or to the antiretroviral treatment in RP patients which partially restores some immune parameters.

Table 6
Associations between IgG/IgG4 levels (extreme quartiles) and Th1/Th2 cytokines polymorphisms

Patients	Parameter	SNP	Location	Calculation mode		
				AF	D/R	G
SP & RP (combined)	**IgG4**	IL6_415	Promoter		0.03	0.02
		IL6_282	Intron 2		0.05	
		IL10_302	Intron 3		0.02	0.02
	IgG	IL10_290	Intron 3		0.05	0.04
	Ratio IgG/IgG4	IL6_415	Promoter			0.05
		IL10_66	Intron 1	0.02	0.03	0.04
		IL10_302	Intron 3		0.05	
SP (alone)	**IgG**	IL10_226	Promoter			0.03
	Ratio IgG/IgG4	IL2_12193	Exon 1	0.05		
RP (alone)	**IgG**	IL10_302	Intron 3	0.04		0.04
		IFNγ_12211	Intron 1			0.04
		IFNγ_12215	Intron 2			0.03
	IgG4	IL6_415	Promoter		0.03	
		IFNγ_12221	Intron 2		0.05	0.01
	Ratio IgG/IgG4	IL6_415	Promoter		0.02	0.009
		IL10_44	Promoter	0.02	0.04	0.03
		IL10_302	Intron 3			0.03
		IFNγ_12221	Intron 2		0.04	0.01

We took again the patients in the extreme n-tiles (n = 2, 3 or 4) for IgG, IgG4 and the ratio IgG4/IgG. We compared for each SNP the genetic distribution of their two alleles between the low and the high n-tile and used a Fisher's exact test to assess if there was a significant difference of distribution. The table presents the SNPs for which a significant difference could be observed in at least two n-tiles. The differences were tested using various genetic models: allelic frequency (AF), dominant (D), recessive (R) and the genotypic distribution (G). The dominant and recessive modes lead to similar p values since the SNPs are bi-allelic.

We compared the biological parameters between SP and RP patients and found that there were clear differences between the two groups as previously reported [26]. First of all, we found a correlation between $CD4^+$ and $CD8^+$ cell counts in RP patients, which has been described previously [27]. This correlation supports the hypothesis of the HIV-1 infection as an exhaustion of $CD4^+$ T cells [28] initially compensated by the proliferation of $CD8^+$ cells to maintain the homeostasis of the ($CD4^{++}$ $CD8^+$) homeostatic parameter [29]. Indeed, in patients at an advanced stage of disease such as RP patients, the $CD4^+$ cells are too crippled to activate the proliferation of $CD8^+$ cells, which translates into a depletion of both cell types. This is in accordance with the important role of $CD4^+$ T cells as helpers of the immune response. We then refined our study by comparing the patients with extreme values (patients located in extreme quartiles) for all the parameters in order to detect more specific correlations. We found that the haematological cell counts were rather well correlated between each other, even in the RP group for which parameters might have been skewed due to antiretroviral treatment. The asymmetries of the matrixes suggest that the IgG and IgG4 levels were dependent on the cell counts. Moreover, we could compare the correlations found between parameters in SP patients with the ones in RP patients. Most major correlations present in the SP population could also be found in the RP population. However, there are still a few differences between these two populations.

Among SP patients, IgG correlated with $CD4^+$ and $CD8^+$ counts and the $\%CD4^+$ (low IgG with higher $CD4^+$ and $\%CD4^+$, and lower $CD8^+$), while no correlation with cell counts was observed in RP patients. For IgG4 levels, a correlation was observed with $CD8^+$ cell counts in SP patients (low IgG4 with higher $CD8^+$ cell count) and with % of $CD4^+$ cells in RP patients (low IgG4 with higher $\%CD4^+$). As previously mentioned, this could be explained by the sufficient level of $CD4^+$ cells in SP subjects making the % of $CD4^+$ cells less informative and the $CD8^+$ cell counts more representative. In RPs, the correlation between $CD4^+$ and $CD8^+$ cells leads to a very sensitive $\%CD4^+$ ratio and that may explain the correlation found with IgG4 levels. Still it does not tell us by which mechanism the production of IgG4 is correlated with the balance between $CD4^+$ and $CD8^+$ cells. This correlation might be caused by other cell types evolving in a similar way to $CD8^+$ cells in AIDS patients.

We studied the distribution of 23 USP patients and 22 SSP patients between the extreme quartiles of each biological parameter. We used these data to establish which parameter would be the best for the prediction of the outcome of the disease among those patients. It turns out that the best predictor for progression to AIDS is the viral load (three USP patients among low VL vs. 11 USP patients among high VL), the best predictor for stability is the $CD4^+$ cell count (two SSP patients among low $CD4^+$ vs. 10 SSP patients among high $CD4^+$), and the best predictor for patient evolution (stability vs. progression to AIDS) is $\%CD4^+$ with a p-value of 0.04. Indeed, the SP patients generally have a low viral load and high $CD4^+$ cell count. We also noticed that high levels of IgG could be associated with future progression to AIDS (three USP patients among low IgG against eight USP patients among high IgG). These results still need to be extended since only 45 SP patients out of 165 had a clear evolution pattern during the 3-years follow-up (22 SSP and 23 USP patients).

Mellors et al. [30] have shown that the viral load was a good predictor of progression to AIDS. However, their patients had a much higher viral load than ours, suggesting that they were at an advanced stage of disease. In another study [31], the viral load was found as a better predictor of progression to AIDS than the CD4+ cell count. This study showed that subjects with a very high viral load (above 10 000 copies/ml) will significantly progress to AIDS compared to subjects with a lower viral load. Our results are not unconsistent with these findings since most of the SP patients we are dealing with have a much lower viral load. These previous studies are overall compatible with our results suggesting that CD4+ percentage might be more discriminative for predicting stability vs. progression at early stages of disease. Indeed, the most recent recommendations suggest to use antiretroviral treatment only when the CD4+ cell decline is clear (CD4+ cell counts below 300/mm^3) or when a real burst in viral load occurs, much higher than the average 250 copies/mm^3 we observe here in the SP patients.

The Th1/Th2 cytokines are known to be involved with the Ig isotypic switch. Therefore, we tested whether some genetic polymorphisms in cytokine genes (IL2, IL4, IL6, IL10, IL13, IFNγ) could be associated with the levels of IgG/IgG4 in the SP and RP populations. We indeed found some significant associations (p-values varying between 0.01 and 0.05) between SNPs mainly in IL2, IL6, IL10, and IFNγ and extreme levels of IgG and IgG4. IL10 gene and more precisely IL10_44, IL10_66 and IL10_302 may play a role in IgG and IgG4 levels.

In conclusion, IgG or IgG4 levels do not seem to be better for predicting disease evolution or disease stage than the usual haematological parameters or viral load. A previous study has found such a predicting power for specific anti-HIV-1 IgG2 levels [32], and may-be the IgG4 directed against HIV-1 epitopes should have been evaluated more specifically in our study. Further studies should also investigate the IgE levels, the role of the percentage of CD4+ cells as a prognosis marker for stability versus progression to AIDS and the possible role of the IL10 in controlling the production of these immunoglobulin in the context of an inflammation such as AIDS.

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