

Eosinophils in HIV Patients Co-Infected by HTLV-1 and/or *Strongyloides stercoralis*: Protective or Harmful Depending on HIV Infection Stage

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Abstract

Polymorphonuclear eosinophils are source of chemokines such as RANTES and IL-16. These chemokines suppress the *in vitro* viral replication of HIV primary strains and so could contribute to the resistance of infection in HIV seronegative patients despite their belonging to risk groups. Hypothesis considering eosinophilia as a protective factor against HIV infection by overexposing RANTES was tested. Therefore we studied the impact of co-infections of human T lymphotropic virus-1 (HTLV-1) and *Strongyloides stercoralis* (Ss), infectious agents potentially eosinophylogenic, on the eosinophilic reaction in HIV patients and on the patients' survival. From 1983 to 1996, 445 HIV infected patients had a follow-up for at least one year of whom 52% developed AIDS, 13% presented with HTLV-1, 15% with Ss co-infection and 23% showed eosinophilia superior to $1.10 \times 9/L$. Our results indicate that eosinophilia provoked by Ss was not altered by HTLV-1. Furthermore the reactive ability of eosinophils was not affected by the drastic decrease of CD4-T observed in HIV patients. HIV patients co-infected by HTLV-1 presented higher amounts of CD4-T as compared to patients only infected by HIV, but no effect on CD8-T and eosinophils amount was observed. The median age superior in HTLV-1/Ss co-infected HIV patients suggested an asymptomatic period lengthened because of later diagnostic and a protective effect of these co-infections. However patients' survival in AIDS phase was neither changed by eosinophilia nor by the HTLV-1 and Ss co-infections.

Keywords: HIV; HTLV-1; *Strongyloides stercoralis*; Eosinophilia; Retroviral interactions

Introduction

Peripheral eosinophils represent less than 1% of total eosinophils, which are mainly tissular cells. As compared to lymphocytes (10^{12}) of which 2% are circulating cells, the total population of eosinophils (10^{10}) is one hundred times lower, but in the progression of HIV infection they become relatively more important.

The relative resistance to the HIV infection of patients belonging to risk groups seems associated to the activities of three β -chemokines CC, namely RANTES, MIP-1 α , MIP-1 β [1]. These chemokines and IL-16 suppress the *in vitro* viral replication of primary strains of HIV [2-4]. Furthermore they block CCR5, the second receptor of primary non-syncytium-inducing or macrophage (M)-tropic HIV expressed at the surface of CD4-T cells and monocytes. Among the chemokines RANTES represents the one showing the most important affinity to CCR5 [4]. *In vitro* activated eosinophils are source of chemoattractants such as RANTES and IL-16 [5-7].

In HIV patients, various co-infections are observed and according to the secreted cytokines, they affect the HIV infection in a favorable or unfavorable manner [8]. In Martinique, HIV-infected patients may be co-infected by HTLV-1 and *Strongyloides stercoralis* (Ss), two endemic infectious agents having an inductive capacity of eosinophilia [9-12]. Here we propose the reactive ability of eosinophils in HIV-infected patients as a relative protective factor against HIV through a high expression of ligand RANTES in eosinophils [5-7]. In order to study this hypothesis, we investigate the role of the co-infections of HTLV-1 and/or Ss on the eosinophilic reaction and the impact of eosinophilia on the survival of HIV-infected patients.

Methods

The definition of eosinophilia retained in this study corresponds to a number of circulating eosinophils equal or superior to $1.10^9/L$. Eosinophils were considered on the one hand by the presence ($>1.10^9/L$) or absence ($<1.10^9/L$) of eosinophilia and on the other hand by the

maximal amount observed during the evolution of HIV infection. Analysis of eosinophilia was realized in terms of the stage of the disease, either at the asymptomatic or the AIDS stage. The presence of Ss was determined by direct detection of rhabditiform larvae in fresh stool samples concentrated by the Baerman funnel technique and/or by direct detection of larvae in bronchoalveolar lavage fluid and gastric fluid. Serum specimens were screened for antibodies to HTLV-1 with an enzyme-linked immunosorbent assay. Positive results were confirmed by Western blot analysis. Subsets of CD4-T and CD8-T cells were analyzed by flow cytometry (FACStar or FACScalibur, Becton Dickinson). Indicated values of CD4-T and CD8-T correspond at the moment when a maximal number of eosinophils was observed at any stage of the disease. Rates of HTLV-1 co-infection and Ss co-infection and eosinophilia were compared for different CD4-T counts either inferior to $0.2.10^9/L$, between $0.2.10^9/L$ and $0.5.10^9/L$ or superior to $0.5.10^9/L$.

Patients

From January 1, 1983 to March 30, 1996, 815 HIV-seropositive subjects were identified in Martinique and followed up at the University Hospital of Fort de France. Of them 378 (46%) developed AIDS. During study period, none patient was treated by antiproteases known to alter the expression of RANTES [13,14]. To analyse the evolution of eosinophils and lymphocytes rates, only patients with a minimal follow-up of one year were included in the study. So, patients were

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investigated by a monthly lymphocyte and eosinophils blood count and by subsets of CD4-T and CD8-T cells. The cohort of patients were also tested for HTLV-1 and Ss infection. Hence HIV-patients involved in this study were separated into four groups: 1) patients only infected by HIV, 2) patients co-infected by HTLV-1, 3) patients co-infected by Ss, 4) patients co-infected by both HTLV-1 and Ss. The groups of patients were described by the following main characteristics: sex, prevalence of HTLV-1 and Ss, eosinophilia (presence or absence) and the correspondent numbers of CD4-T as well as CD8-T, the incidence of AIDS, patients' age at the moment of HIV infection diagnosis, the age of progression to AIDS, and age of maximal amount of eosinophils and of death.

Statistical analysis

Patients' characteristics: HTLV-1 infection, Ss infection, biological characteristics and the progression to AIDS were studied in terms of age and sex using univariate analysis. We used Chi-square test, Shapiro-Wilk test to verify normal distribution for the quantitative parameters and non-parametric Kruskal-Wallis test to inter-group comparison. Patients in whom Ss infection was not evaluated were excluded from analysis. The number of cases included (N) varied as a result of missing values.

Longitudinal analysis: The study was applied to a cohort of patients having a minimal follow-up of one year between 1983 and 1996. Patients' age was taken into account for three irreversible events: 1) age of HIV infection diagnosis, 2) progression to AIDS, 3) death at any clinical stage of the disease. Ages and intervals between the different events are expressed in years. Survival is defined as the time in years from the known year of either HIV diagnosis, entrance in AIDS stage (CD4-T count $< 0.2.10^9/L$), or maximal amount of eosinophils, until the occurrence of death. The influence of explanatory parameters such as sex, HTLV-1 infection, Ss infection and eosinophilia was investigated. Survival analysis was analyzed with actuarial method using March 30, 1996, as the study endpoint. The log-rank test was applied in order to compare the groups and to assure that during the follow-up there was no crossing-over of the curves obtained by the actuarial method. The Cox proportional hazards model was applied to appreciate the associated risk of the explanatory variables and the occurrence of each irreversible event.

Multivariate analysis: A model of logistic regression was used to study the influence of HTLV-1 and Ss on eosinophilia. First, univariate models were applied to evaluate the crude odds-ratios of the explanatory parameters and their 95% confidence intervals. Furthermore for the multivariate analysis only parameters significantly associated to eosinophilia were selected by using a limit inferior or equal to 25 percent. Finally interactions between the different parameters and potential confounders such sex were analyzed. Results are first presented for the whole cohort of patients, then for each group of patients either at the AIDS stage or the asymptomatic stage and finally for each CD4-T amount either inferior to $0.2.10^9/L$, between $0.2.10^9/L$ and $0.5.10^9/L$ or superior to $0.5.10^9/L$. Statistical significance was obtained by a p-value inferior to 0.05. Statistical analyses were performed with statistical software STATA 7.0 (StataCorp, College Station, TX).

Results

During the period of study 445 patients were selected on the basis of a minimal follow-up of one year. Sex ratio was 2.3 (310M/135F). Two hundred and thirty one patient (51.9%) progressed to AIDS, 181 (40.7%) died and 27 patients (6.1%) were lost to follow-up. Fifty-eight

patients (13%) were co-infected by HTLV-1 and 55 patients (14.9%) of 368 patients tested were co-infected by Ss. One hundred and four patients (23.4%) showed eosinophilia superior to $1.10^9/L$. The median age of HIV infection diagnosis, progression to AIDS, maximal amount of eosinophils and death were respectively of 35, 40, 38, 41 years. As the continuous variables did not follow the normal distribution, biological characteristics and the prevalence of infections were expressed as median values and percentages. All the parameters, i.e. CD4-T cells counts, eosinophilia, prevalence of HTLV-1 infection, Ss infection and the proportion of patients at the AIDS stage, except the amount of CD8-T and the percentage of death, showed a significant difference as analyzed in terms of sex (Table 1). Therefore, results are presented according to sex. The prevalence of Ss infection was significantly higher in patients infected by HTLV-1 as compared to non-infected patients (14/44 versus 41/324, $p=10^{-3}$).

The median age of HIV diagnosis and progression to AIDS and the median age of maximal amount of eosinophils were lower in patients who were not infected by HTLV-1 as compared to the other groups. These findings were independent of the clinical situation and of sex and they were observed despite the variable number of patients. Otherwise the median age of diagnosis time and progression to AIDS was higher in patients only infected by HTLV-1 as compared to patients only infected by Ss. The same difference was noted at the AIDS stage of disease (Table 2a-2c).

Correlation between HTLV-1 infection, Ss infection and amounts of CD4-T, CD8-T and eosinophils

Significantly higher values of CD4-T cells were observed in patients (whole cohort) without eosinophilia and also in patients co-infected by HTLV-1 or Ss as compared to those not co-infected. In opposition no significant difference could be observed concerning CD8-T cells under any condition (Table 3a). At the AIDS stage only HTLV-1 and Ss infections were associated with higher values of CD4-T; the amounts of CD8-T cells did not manifest any significant difference (Table 3b). At the asymptomatic stage, only Ss infection was associated with higher values of CD4-T (Table 3c). Classification of patients by the amount of CD4-T cells either inferior to $0.2.10^9/L$, between $0.2.10^9/L$ and $0.5.10^9/L$ or superior to $0.5.10^9/L$ revealed that the HTLV-1 infection demonstrated a significantly higher number of patients with CD4-T

Variables	Male (N=310)	Female (N=135)	p-value
CD4 (median) x $10^9/L$	0.128	0.211	0.0012
CD8 ((median) x $10^9/L$	0.762	0.695	NS
Maximal amount of Eosinophils* (median) x $10^9/L$	0.628	0.284	10^{-4}
Variables	N (%)	N (%)	
Eosinophilia ($>1.10^9/L$)	90 (29)	14(10.4)	10^{-4}
HTLV-1+	32 (10.3)	26 (19.3)	0.013
Ss+ **	48 (18.3)	7 (6.7).	6.10^{-3}
AIDS	171 (55.2)	60 (44.4)	0.038
Death	134 (43.2)	47 (34.8)	NS

* Observed during the evolution of HIV infection
Ss: *Strongyloides stercoralis*, N: number of patients, NS=no significant, critical level: $p < 0.05$

** Patients in whom the Ss infection was not evaluated were excluded (male:262, female: 135).
AIDS stage is defined by CD4-T count $< 0.2.10^9/L$.

Table 1: Comparison of features of HIV patients, according to sex, diagnosed between 1983 and 1996 with a follow-up > one year (whole cohort) (Kruskal-Wallis test or Chi2 test for %)

cells superior to $0.5.10^9/L$ (Table 4). The proportion of patients with eosinophilia was higher in the group of patients who demonstrated a CD4-T count inferior to $0.2.10^9/L$ as compared to the other groups (Table 4).

Group of patients -whole cohort*	N	Age of HIV diagnosis [x,y,z]**	N	Age of AIDS stage [x,y,z]**	N	Age of death
HTLV- Ss-	283	[26,33,43]	169	[30,37,45]	131	[32,39,50]
HTLV+ Ss-	30	[42,52,61]	16	[45,53,63]	10	[46,57,65]
HTLV- Ss+	41	[32,38,47]	29	[35,41,49]	20	[42,43,57]
HTLV+Ss+	14	[38,48,55]	10	[38,48,55]	8	[42,55,57]
		p=0,0001		p=0,0001		p=0,0061
Male						
HTLV- Ss-	203	[27,34,43]	123	[31,38,45]	96	[33,39,49]
HTLV+ Ss-	13	[47,51,57]	8	[49,53,65]	4	[40,49,59]
HTLV- Ss+	36	[31,36,45]	25	[35,40,48]	17	[37,43,50]
HTLV+Ss+	12	[35,43,54]	9	[38,44,55]	7	[41,54,57]
		p=0.001		p=0.0043		NS
Female						
HTLV- Ss-	80	[25,30,42]	46	[29,34,45]	35	[30,37,51]
HTLV+ Ss-	17	[40,55,61]	8	[42,54,63]	6	[47,63,66]
HTLV- Ss+	5	[44,47,54]	4	[42,48,56]	3	[42,46,55]
HTLV+Ss+	2	52,55	1	55	1	
		p=5.10 ⁻⁴		p=0.03		p=0.05

* Patients in whom the Ss infection was not evaluated were excluded
 ** x,y,z : Percentile [25, 50, 75]
 Ss: *Strongyloides stercoralis*
 AIDS stage is defined by CD4-T count < $0.2.10^9/L$.
 N: number of patients, NS: no significant, critical level: p < 0.05

Table 2a: Age of HIV patients at the moment of three irreversible events (HIV diagnosis, AIDS stage, death) in the four groups in whole cohort* (Univariate analysis).

Group of patients AIDS*	N	Age of HIV diagnosis [x,y,z]**	N	Age of AIDS stage [x,y,z]**	N	Age of death [x,y,z]**
HTLV- Ss-	169	[26,34,43]	169	[30,37,45]	115	[31,38,47]
HTLV+ Ss-	16	[41,52,61]	16	[45,53,63]	10	[46,57,65]
HTLV- Ss+	29	[32,37,47]	29	[35,41,49]	18	[37,43,56]
HTLV+Ss+	10	[38,47,55]	10	[38,48,55]	8	[42,55,57]
		p=0,0002		p=0,0001		p=0,003
Male						
HTLV- Ss-	123	[27,35,43]	123	[31,38,45]	84	[32,39,61]
HTLV+ Ss-	8	[48,52,64]	8	[49,53,65]	4	[40,49,59]
HTLV- Ss+	25	[32,35,46]	25	[35,40,48]	16	[37,43,51]
HTLV+Ss+	9	[38,42,55]	9	[38,44,55]	7	[41,54,57]
		P=0.01		P=0.0043		NS
Female						
HTLV- Ss-	46	[26,29,42]	46	[29,34,45]	31	[30,37,51]
HTLV+ Ss-	8	[39,52,61]	8	[42,54,63]	6	[47,63,66]
HTLV- Ss+	4	[40,46,51]	4	[42,48,56]	2	42,46
HTLV+Ss+	1	52	1	55	1	56
		p=0.03		p=0.03		NS

*Patients in whom the Ss infection was not evaluated were excluded
 ** x,y,z: Percentile [25, 50, 75]
 Ss: *Strongyloides stercoralis*
 The entrance in AIDS stage is defined by CD4-T count < $0.2.10^9/L$.
 N: number of patients, NS: no significant, critical level: p < 0.05

Table 2b: Age of HIV patients at the moment of three irreversible events (HIV diagnosis, AIDS stage, death) in the four groups in AIDS patients (Univariate analysis).

Groups of patients*	Whole cohort* N	Age of maximal amount eosinophils [x,y,z]**	AIDS patients* N	Age of maximal amount eosinophils [x,y,z]**
HTLV- Ss-	283	[31,36,46]	169	[32,38,45]
HTLV+ Ss-	30	[46,55,65]	16	[44,55,65]
HTLV- Ss+	41	[36,41,49]	29	[35,42,49]
HTLV+Ss+	14	[41,50,57]	10	[42,50,56]
all	368	p=0,001	224	P=0,001

*Patients in whom the Ss infection was not evaluated were excluded
 ** x,y,z: Percentile [25, 50, 75]
 Ss: *Strongyloides stercoralis*
 The entrance in AIDS stage is defined by CD4-T count < $0.2.10^9/L$.
 N: number of patients, critical level: p < 0.05

Table 2c: Age of HIV patients at the moment of maximal amount of eosinophils in whole cohort* and AIDS patients.

Event		N	CD4 median**	p-value	N	CD8 median**	p-value
Eosinophilia*	yes	88	0.091	0.0034	88	0.687	NS
	no	309	0.187		300	0.739	
HTLV-1	yes	46	0.241	0.0049	44	0.783	NS
	no	351	0.143		344	0.727	
Ss	yes	47	0.175	4.10⁻⁴	47	0.755	NS
	no	279	0.108		271	0.652	

*yes: > $1.10^9/L$, no: < $1.10^9/L$
 ** x $10^9/L$
 Ss: *Strongyloides stercoralis*
 N: number of patients, NS: no significant

Table 3a: Comparison of CD4-T and CD8-T rates in terms of either eosinophilia (> $1.10^9/L$), HTLV-1 infection or Ss infection in the whole cohort (Kruskal-Wallis test, critical level: p < 0.05)

Event		N	CD4 median**	p-value	N	CD8 median**	p-value
Eosinophilia*	yes	59	0.025	NS	59	0.566	NS
	no	139	0.047		132	0.531	
HTLV-1	yes	23	0.164	NS	21	0.423	NS
	no	175	0.038		170	0.536	
Ss	yes	33	0.094	NS	33	0.653	NS
	no	161	0.035		154	0.521	

*yes: > $1.10^9/L$, no: < $1.10^9/L$
 ** x $10^9/L$
 Ss: *Strongyloides stercoralis*
 N: number of patients, NS: no significant

Table 3b: Comparison of CD4-T and CD8-T rates in terms of either eosinophilia (> $1.10^9/L$), HTLV-1 infection or Ss infection in AIDS patients (Kruskal-Wallis test, critical level: p < 0.05).

Event		N	CD4 median**	p-value	N	CD8 median**	p-value
Eosinophilia*	yes	29	0.346	NS	29	0.976	NS
	no	170	0.326		168	0.931	
HTLV-1	yes	23	0.465	NS	23	1.061	NS
	no	175	0.321		174	0.931	
Ss	yes	14	0.476	NS	14	1.033	NS
	no	118	0.289		117	0.88	

*yes: > $1.10^9/L$, no: < $1.10^9/L$,
 ** x $10^9/L$.
 Ss: *Strongyloides stercoralis*
 N: number of patients, NS: no significant

Table 3c: Comparison of CD4-T and CD8-T rates in terms of either eosinophilia (> $1.10^9/L$), HTLV-1 infection or Ss infection in asymptomatic patients (Kruskal-Wallis test, critical level: p < 0.05).

Event		N	< 0.2 CD4* %	N	0.2<CD4<0.5* %	N	> 0.5 CD4**%	p-value
Eosinophilia**	yes	60	68.2	15	17	13	14.8	0.01
	no	165	53.4	84	27.2	60	19.4	
HTLV-1	yes	19	41.3	11	23.9	16	34.8	0.02
	no	190	58.7	88	25.1	57	16.2	
Ss	yes	27	57.5	10	21.3	10	21.3	NS
	no	183	64.9	60	21.5	38	13.6	

N: number of patients, NS: no significant, critical level: p< 0.05

**yes: > 1.10⁹/L, no: < 1.10⁹/L

Ss: *Strongyloides stercoralis*

Table 4: Comparison of proportions of patients with eosinophilia (>1.10⁹/L), HTLV-1 infection or Ss infection (Chi2 test) in each CD4 class*: either inferior to 0.2.10⁹/L, between 0.2.10⁹/L and 0.5.10⁹/L or superior to 0.5.10⁹/L. CD4-T count < 0.2.10⁹/L defines the entrance in AIDS stage.

Studied variables	Whole cohort* (N = 368)	Eos>1.10 ⁹ /L		Univariate analysis			Multivariate analysis Final model		
		N	%	OR ^a crude	95%CI	p-value	OR ^a adjusted	95%CI	p-value
Sex						<10 ⁻³			<10 ⁻⁴
Female	104	12	11.5	1.00			1.00		
Male	264	84	31.8	3,58	[1,86 ; 6,89]		2.26	[1,66 ; 6,23]	
Ss						<10 ⁻³			<10 ⁻⁴
No	313	70	22.4	1.00			1.00		
Yes	54	26	48.1	3,11	[1,72 ; 5,63]		2.68	[1,47 ; 4,91]	
HTLV						0.102			
No	324	86	26.5	1.00					
Yes	44	10	22.7	0.81	[0,39 ;1,72]				

* Patients in whom the infection of Ss was not evaluated was excluded

OR: odds ratio, CI : confidence interval

The p value was calculated with the use of an adjusted Cox proportional hazards model

Ss: *Strongyloides stercoralis*, eos: eosinophils

N: number of patients, critical level: p< 0.05

Table 5: Coinfluence of HTLV-1 and Ss on eosinophils (Eos) in HIV patients (Logistic regression).

Co-influence of HTLV1 and Ss on amount of eosinophils (logistic regression)

With regard to eosinophils counts and eosinophilia (>1.10⁹/L) prevalence, no significant difference was revealed when patients co-infected by HTLV-1 and Ss were compared to those only infected by Ss (6/13 versus 10/20, p=0.1). Otherwise the risk for eosinophilia was higher in male (Odds ratio of 2.26, 95% confidence interval: 1.66;6.25) and in patients infected by Ss (Odds ratio of 2.68, 95% confidence interval: 1.47;4.91) (Table 5).

Analysis of survival

In asymptomatic patients the median survival time was not reached at study endpoint. The different variables studied i.e. Ss infection, HTLV-1 infection and eosinophilia were not related to death in opposition to the AIDS stage. Indeed, the different actuarial curves of survival (data not shown) did not show any significant difference for these parameters. The Cox model analysis confirmed the analysis by the actuarial method and showed a 9.4 times higher relative risk to death in the AIDS stage as compared to the other patients. In the AIDS stage, analysis showed that others studied variables, sex, HTLV-1 or Ss infections, eosinophilia, were not related to death in studied population (Table 6).

Discussion

In HIV infection a moderate eosinophilic reaction is commonly observed without identifying a specific etiologic agent [15,16]. The

increase of blood eosinophils as well as the augmentation of IgE accompanying often skin diseases are observed in the advanced stage of HIV infection and thus are considered of poor prognosis [17-19]. In opposition, the *in vivo* increased production of IL-4 and IgE as well as eosinophils increased amount noted in patients in the asymptomatic stage of HIV infection is in agreement with a protector effect of the IgE/eosinophil system [20,21]. Otherwise, activated eosinophils can generate a number of toxic substances (eosinophil peroxidase, hydrogen peroxide and halide ions) which have lytic effects on HIV [22]. On opposite, *in vitro* HIV-infected eosinophils do not survive and they die by apoptosis or necrosis [23]. However, these findings are not informative about eosinophils' impact on the progression of HIV infection.

In this study, we analyzed the protective or detrimental role of pathogens such as HTLV-1 and/or Ss on the progression of HIV infection through their potential function as inductor or modulator of the eosinophilic reaction. Our results suggest that the IgE/eosinophil system should operate differentially in function of the stage of the HIV infection. In the asymptomatic phase, the viral NSI/M-tropic strains use preferentially the CCKR5 receptor. RANTES, a strong chemoattractant for CD4/CD45RO T cells [24], secreted by eosinophils [2,7], should block the HIV-receptor CCK-R5 of adjacent CD4-T cells; by this way eosinophils (induced by Ss) should be a limiting factor for HIV infection. Otherwise, the observations of the decrease of RANTES in blood in "progressors as compared to "non-progressors on the one hand

Studied variables	Whole cohort (N = 445)	Death		Univariate analysis			Final model		
		N	%	RR crude	95%CI	p-value	RR crude	95%CI	p-value
AIDS						<10 ⁻⁴			<10 ⁻⁴
No	214	21	10	1,00			1,00		
Yes	231	160	69	9,42	[5,97;14,88]		9,42	[5,97;14,88]	
Sex						<10 ⁻³			
Female	135	47	35	1,00					
Male	310	134	48	1,48	[0,97; 2,26]				
Eosinophils >1.10⁹/L						0.110			
No	342	129	38	1,00					
Yes	103	52	50	1,30	[0,94 ; 1,79]				
HTLV						0.069			
No	384	156	41	1,00					
Yes	56	25	45	1,48	[0,97 ; 2,26]				
Ss						0.09			
No	313	144	46	1,00					
Yes	55	30	55	1,41	[0,95; 2,09]				

RR: estimated Relative Risk, CI: confidence interval. The RR are calculated at the end of the study (study endpoint march 30, 1996).

N: number of patients, Ss: *Strongyloides stercoralis*

Table 6: Results of the multivariate analysis of relative risk to death according to HTLV-1 and Ss infections, eosinophils count, AIDS phase and sex using Cox regression model (p-value at 25%).

and the increase of RANTES in patients treated by protease inhibitor on the other hand also suggest a role for RANTES in the control of HIV infection [13].

So, in the asymptomatic phase of the disease, Ss infection inductor of stimulated eosinophils that produces RANTES would be unfavorable to HIV [22]. In the advanced AIDS disease, the period of the SI/T tropic viral strains whose preferential receptor is CXCR4, activated eosinophils sensitive to the HIV infection die and so they contribute to the progression of the disease [23,25,26].

HTLV-1 influence on eosinophils and CD4-T and CD8-T counts

Influence of HTLV-1 on blood eosinophils amount: Our data indicate that HIV patients co-infected by HTLV-1 do not present significantly different values of circulating eosinophils as compared to patients infected only by HIV (table V). Otherwise, in opposition to skin diseases related to HIV [17-19], those observed in HTLV-1 infection in the course of the adult T-cell leukaemia/lymphoma (ATL) [27] or the infectious dermatitis related to HTLV-1 [28], do not show a cutaneous infiltration of eosinophils or blood eosinophilia. So, these data suggest that HTLV-1 does not modify eosinopoiesis. However, this proposal is in opposition to the others findings [29-31]. This difference could be explained, first by the means of the recruitment of the patients, second by the non considering of parasitic infections, in particular the helminthic infections and third by a too low threshold retained for the definition of eosinophilia.

Influence of HTLV-1 on CD4 and CD8 T-cells: Several studies have shown that in HTLV-1 coinfecting HIV-patients the number of HTLV-1+CD4-T [32-35] or HTLV-1+CD8-T is increased [35,36]. In agreement with these studies, we demonstrate that carriers of both HIV and HTLV-1 present a significantly higher level of CD4-T lymphocytes as compared to patients infected only by HIV. A spontaneous activation and proliferation of lymphocytes in the presence of HTLV-1 may cause this finding [37], especially as co-infected by Ss [38]. On the other hand, in our study, HTLV-1 co-infection does not alter the amount of peripheral CD8-T cells under any conditions (Table 3 a,b,c).

Reactive ability of eosinophils in HIV patients co-infected with Ss and HTLV-1: Eosinophilia and an increased production of IgE (markers of a Th2 response) are common features of helminthic infections. In HIV-infected patients, preservation and even an expansion of the eosinophilic lineage are observed [19-21]. According to our study, the frequency of eosinophilia and its level in HIV patients coinfecting by Ss or by both Ss and HTLV-1 are the same as those commonly observed in patients only infected by Ss or by both Ss and HTLV-1 and not by HIV, even when the levels of CD4-T and CD8-T are low. Thus it appears that neither HIV infection, nor HTLV-1 infection does affect the beneficial reactive ability of the eosinophils against helminthic parasites, in particular Ss. In line with this, in Martinique, which is an endemic area for HIV, HTLV-1 and Ss, HIV-infected patients in opposition to those infected only by HTLV-1 are less often infected by Ss and the hyperinfection syndrome of Ss is uncommon [39].

Independence of the eosinophilogenic CD4-T and CD8-T populations: Eosinophilia detected in HIV-infected patients, in particular in those co-infected by Ss, gives evidence that either CD4-T clones or the cytokines controlling the eosinopoiesis are not affected by the HIV infection or that this function is assured by CD4-CD8+ T clones. Several reports are in agreement with this hypothesis insofar as CD8-T-lymphocytes would mime a Th2 function in producing IL-4, IL-5 [40,41]. The degeneration of the cellular immunity relative to the CD4/CD8/Cytotoxic-T lymphocyte effector system would slow down and would partially be compensated by the amplification of the CD8-T dependent eosinophil/IgE effector system [42]. Actually our results do not reveal any correlation as concerns the level of CD4-T, CD8-T cells and eosinophilia. In particular, when the level of CD4-T cells is inferior to 0.2.10⁹/μL, CD8-T cells no more decrease and eosinophilia is independent of level of CD4-T and CD8-T cells; except for the whole cohort no eosinophilia was significantly associated with a higher level of CD4-T cells (table 3a).

Impact of eosinophilia on clinical progression in HTLV-1 co-infected HIV patients: Evidence of eosinophilia and increased serum levels of IgE observed in the advanced clinical phase of HIV infection suggest that these biological parameters can be considered of poor prognosis [17-19]. Several studies have shown that in HTLV-

1 coinfecting HIV-patients the clinical progression of the disease is accelerated when compared to patients only infected by HIV [32,43-45]. However other studies have not confirmed the observation that HTLV-1 modulates the clinical progression in HIV infected patients [46,47]. Our study reveals in dually HIV/HTLV-1 infected patients the same level of eosinophils as compared to patients only infected by HIV (table 5). Hence, during the AIDS phase in dually infected HIV/HTLV-1 patients, the progression of the disease should be equivalent. Nevertheless this hypothesis is in opposition to several studies [44-46]. Otherwise, the relative resistance to HIV of patients belonging to a risk group (multiple high-risk sexual exposures) unfortunately does not give any indication neither of their parasitic and retroviral co-infections nor on their level of eosinophils in peripheral blood [1].

Whatever the reason of eosinophilia, in our study HIV patients with eosinophilia and HIV patients either co-infected by HTLV-1 or by both HTLV-1 and Ss presented a median age superior to the one of patients only infected by HIV (Table 2 a,b,c). This observation which is consistent with other reports [39,46] implies a slower progression in HTLV-1/Ss co-infected patients to AIDS disease and hence are later diagnosed. The beneficial eosinophilia, induced by chronic helminthic infections such as Ss or others helminths, elaborating an excess of chemokines such as RANTES, IL-16 and MIP-1 α partakes perhaps to the observed protection or at least to the delayed AIDS disease [39,48,49].

Nevertheless analysis of the global survival, survival curves (data not shown) and associated risk of the explanatory parameters after the AIDS phase do not show any significant difference in AIDS patients co-infected by HTLV-1 and/or Ss and non-co-infected patients (Table 6) : the length of AIDS stage were not modified by Ss/HTLV-1 coinfections

HIV and HTLV-1 infections alter the IL-2/IL-2R system. So, HIV infected patients exhibit a failure in producing IL-2 and its receptor [20,50]. At the contrary, HTLV-1 infection induces in asymptomatic carriers a variable expression of IL-2 (Th1 profile) and its receptor [51,52]. Furthermore, a prolonged administration of small quantities of IL-2 in HIV patients induces the expansion of NK effector cells and of eosinophils without causing first any toxicity, second an enhancement of the viral charge and third an appearance of opportunistic infections in a follow-up of fifty months [53]. So, the HTLV-1 by inducing a low IL-2 expression and restoring the NK activity in the asymptomatic stage of HIV infection would arrest the progression to the AIDS disease. *In vivo* HTLV-1 by means of hampering the Th2 profile [54] would induce a decrease of eosinophil activation and hence would limit their HIV infection.

Conclusion

Our study suggests that eosinophils exert different functions according to HIV infection phase: protective during the asymptomatic phase, harmful during AIDS phase. Overall survival of HIV patients co-infected by HTLV-1 or Ss is lengthier than the one of patients only infected by HIV. Our study suggests a lengthening of the asymptomatic phase; however, co-infections by Ss or HTLV-1 do not modify the survival time of AIDS phase. Otherwise, our observations reveal the problem of the control of retroviral and helminthic co-infections which disturb the complex and fragile balance of the cytokines and their receptors expressed on lymphocytes and eosinophils. Eosinophils in elaborating cytokines either of type Th1 or type Th2 participate to the general control of the response of T cells [55-57]. So, they could modulate the evolution of HIV infection, especially in the asymptomatic stage.

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