

Original article

Skin reactivity to aeroallergens is reduced in human T-lymphotropic virus type I-infected healthy blood-donors (asymptomatic carriers)

Background: A type 2 immune response, characterized by high levels of interleukin-4 and immunoglobulin E synthesis is a hallmark of respiratory allergic diseases. Individuals infected with human T-lymphotropic virus type I (HTLV-I) virus have spontaneous T-cell proliferation and increased interferon γ production, which are immunological functions associated with a type 1 immune response.

Objective: To determine the frequency of asthma and rhinitis symptoms and immediate skin reactivity to aeroallergens in HTLV-I infected individuals, compared with noninfected subjects.

Methods: Cross sectional study of 101 HTLV-I infected and 101 control uninfected blood donors, assessed by enzyme-linked immunosorbent assay and Western blot assays. The subjects were age and sex-matched, identified as presenting allergy history by questionnaire, which was complemented by a complete clinical examination and skin prick tests for aeroallergens.

Results: The frequency of atopy was lower in infected than uninfected subjects, 14.9 and 29.7% ($P = 0.017$), respectively. Skin reactivity to *Dermatophagoides pteronissynus*, *Dermatophagoides farinae* and *Blomia tropicalis* were the most frequently observed among all the tested antigens in both groups. Skin reactivity to histamine was also reduced in the infected individuals compared with uninfected subjects (medians 4.0 vs 5.0, respectively; $P < 0.0001$). Infection by HTLV-I was found to be a factor of protection to atopy (RP 0.44; $P = 0.005$).

Conclusions: The HTLV-I infection reduces the frequency of respiratory allergy and skin reactivity to aeroallergens.

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Human T-lymphotropic virus type I (HTLV-I) is a retrovirus highly prevalent in certain endemic areas such as Salvador, Bahia (Northeastern Brazil) where 1.35% of blood donors have circulating antibodies against this virus (1). The HTLV-I infects CD4⁺ and CD8⁺ T cells and establishes persistent infection (2). Individuals infected with HTLV-I have spontaneous T-cell proliferation and produce high levels of interleukin (IL)-2 and interferon (IFN) γ , which are immunological functions associated with a type 1 of immune response (2–3).

Cytokines regulate both the initiation and the maintenance of immune responses against foreign antigens. The balance between Th1 and Th2 cells plays a major role in immunity and hypersensitivity reactions in several

diseases (4–5). Atopy is characterized by an immune system that is biased to type 2 activation. Th2 cells secrete IL-4, which stimulate B-cell proliferation and differentiation to produce immunoglobulin (Ig)E antibodies (6). Broadly stated, naturally occurring infections and microbial exposures might reduce the likelihood of the development of asthma and allergic diseases (4–5).

We have previously demonstrated that HTLV-I infections can suppress type 2 immune response, through suppression of IL-4 production and IgE, reducing immediate skin reactivity in patients co-infected with helminthes (7–9). However, there are evidences that Th2 cells might still be activated during HTLV-I infection despite strong polarization towards type 1 immune response, as we have observed the occurrence of asthma and high levels of IgE has been detected in patients with HTLV-I (10). The viral protein *tax* has been implicated in promoting IL-4 expression (11). In addition, IL-5 has been shown in PBMC supernatants from HTLV-I

Abbreviations: EDSS, Expanded Disability Status Scale; HTLV-I, human T-lymphotropic virus type I; HAM/TSP, HTLV-I associated myelopathy/tropical spastic paraparesis; OR, Odds ratio; PR, Prevalence ratio.

carriers (3). Therefore, the relationship between HTLV-I infection and immune response in allergic disorders, which is of great interest, could not be clearly anticipated. The aim of our study is to determine the prevalence of respiratory allergic diseases and skin reactivity to aeroallergens in individuals infected with HTLV-I and compares it with matched control healthy subjects.

Methods

Patients

In this cross-sectional study, we compared 101 HTLV-I infected and 101 uninfected blood donors recruited from the two major blood banks in Salvador-Bahia-Brazil (Hemocentro da Bahia and Serviço de Transfusão de Sangue), followed up in a specialized outpatient clinic at a University Hospital. All the subjects were age and sex-matched. The University Hospital Medical Ethics Committee approved this study, and all participants gave written informed consent.

HTLV-I serologic tests

All patients were assessed by enzyme-linked immunosorbent assay (Cambridge Biotech Corporation, Worcester, MA, USA) and Western blot assays (Genelabs, Singapore). Additionally all sera were screened for HIV, syphilis, hepatitis B, and hepatitis C.

History and physical examination

At the entry visit, a general health questionnaire was administered to all subjects and their history of atopy was assessed (12). Standardized initial screening examinations were performed on all subjects to evaluate upper and lower airways, skin, and lymph nodes. A complete neurological exam searching for mild manifestations of HTLV-I-related myelopathy was performed and the Expanded Disability Status Scale (EDSS) determined (13). Subjects presenting signs of myelopathy were excluded. Afterwards, skin prick tests (SPT) to aeroallergens and spirometry (14) were carried out. None of the individuals was taking antiallergic, antidepressant or immunosuppressive medicines that might interfere with SPT and they had not been given immunotherapy. All individuals were submitted to fecal analysis by means of Hoffman and Baermann methods and excluded in case of any positive stool sample to helminthes.

Skin prick test

Skin prick tests were performed on the right forearms of all individuals using a panel with 10 relevant local allergens: *Dermatophagoides pteronissynus* (Der p), *Dermatophagoides farinae* (Der f), *Blomia tropicalis* (Blo t), *Aspergillus fumigatus* (Asp), *Cladosporium herbarum* (Clad), *Periplaneta americana* (Perip), *Blatella germanica* (Blat), dog and cat epithelia (Alk Abello, Denmark) according to standardized technique (15). Histamine (1/1000) and saline were used as positive and negative controls, respectively. To avoid possible circadian differences in skin reactivity, skin tests were done between 9 and 11 AM. The size of the reaction was determined by measuring the perpendicular diameters of the papulae, then halving the sum ($d1 + d2/2$). The skin reaction was considered positive after 20 min whether the mean diameter was ≥ 3 mm in the presence of positive histamine reaction and absence of reaction to saline control.

Atopy was defined as history of allergic respiratory disease in the preceding 12 months and SPT positive for at least one antigen tested.

Statistical analysis

Statistical analysis was performed using the software Statistical Package of Social Sciences (SPSS®) 9.0 for Windows®. The independent *t*-test was performed to analyze differences between means. Nonparametric tests such as Mann-Whitney *U*-test and Kruskal-Wallis were used to compare groups when a normal distribution could not be assured. Fisher's exact test was chosen to compare proportions. Prevalence ratios were used to compare the risk for having positive skin reactions to allergens in HTLV-I infected vs uninfected subjects. Binary logistic regression was used to predict factors associated to skin reactivity to allergens (16). The Odds ratio obtained was turned into prevalence ratios by means SAS program version 8.1 for Windows® (17). At a $P \leq 0.05$ differences were considered statistically significant.

Results

Two hundred and two patients were evaluated in this study (92 males and 110 females; age range 20–59 years). All of them scored EDSS = 0 (zero). The individuals were predominantly black (43.1%) and nonsmokers (72.8%). Family history of atopy was detected in 78 (38.6%) subjects and atopy was diagnosed in 45 (22.3%) individuals.

Clinical characteristics

Table 1 summarizes the main data collected. The HTLV-I infected and uninfected individuals were comparable concerning age, gender and racial group distribution. Family history of atopy was not significantly different between infected and uninfected groups (37.6% vs 39.6%; $P = 0.89$). The frequency of atopy was found to be lower

Table 1. Demographic data and history of atopy in human T-lymphotropic virus type I (HTLV-I) infected individuals (asymptomatic carriers) and in HTLV-I uninfected controls

	Infected (<i>n</i> = 101)	Uninfected (<i>n</i> = 101)	<i>P</i> -value
Gender (M/F)	46/55	46/55	1.00
Age in years (mean)	35.2	35.5	0.24
Racial group (%)			0.57
Black	32 (31.7)	39 (38.6)	
Mullatos	47 (46.5)	40 (39.6)	
White	18 (17.8)	20 (19.8)	
Other	4 (4.0)	2 (2.0)	
Number of siblings (median)	5	6	0.009
Blood transfusion (%)	10 (9.9)	0 (0.0)	0.002
Family history of atopy (%)	38 (37.6)	40 (39.6)	0.89
Personal atopy (%)	15 (14.9)	30 (29.7)	0.017

Gender: M, male, F, female; atopy: frequency of asthma and allergic rhinitis in the preceding 12 months and skin prick test positive to one allergen, at least.

in infected than uninfected subjects, 14.9 and 29.7% ($P = 0.017$), respectively. This difference represents a 50% reduction in atopy in the HTLV-I infected group. In this study, the majority of atopic subjects had rhinitis. There were 15 of 101 (14.8%) individuals with allergic rhinitis within the HTLV-I positive and 24 of 101 (23.7%) in the control group. Only 12 of 202 (5.9%) subjects had allergic asthma, seven (3.46%) of them associated to rhinitis. There were five (4.95%) individuals with asthma within the HTLV-I positive and seven (6.9%) in the control group. Asthma was associated to rhinitis in seven of 12 (58.3%) subjects, four within HTLV-I-infected and three within control group.

Skin reactivity

Skin reactivity to *Der p*, *Der f* and *Blo t* were the most frequently observed among all tested allergens in both groups (Fig. 1). The majority of subjects sensitized to *Der p* were also sensitized to *Der f* and in lesser extension to *Blo t*. In order to identify whether not only the frequency but also the magnitude of the skin reactivity to allergens were different in the two groups the sum total of diameters of the skin test reactions was calculated as follows [SPT Sum = dAg1 + dAg2 + dAg3...dAg10] in all subjects. We found that SPT sum was lower in infected than uninfected group (medians 0 vs 3.0 respectively; $P = 0.005$) (Fig. 2). Unexpectedly, skin reactivity to histamine was reduced by 20% in the infected individuals compared to uninfected subjects (medians 4.0 vs 5.0, respectively; $P < 0.001$) as depicted in Fig. 3. Two HTLV-I infected subjects did not react to histamine (1.98%).

In a logistic regression model, predictive variables for skin reactivity to allergens such as serologic status, gender, family history of atopy and reactivity to histamine were tested. Infection by HTLV-I was considered a factor of protection to atopy (RP 0.44; $P = 0.005$). Higher prevalence ratios for development of positive skin reactions were found among females, subjects with a family history of atopy and were associated with reactivity to histamine (Table 2).

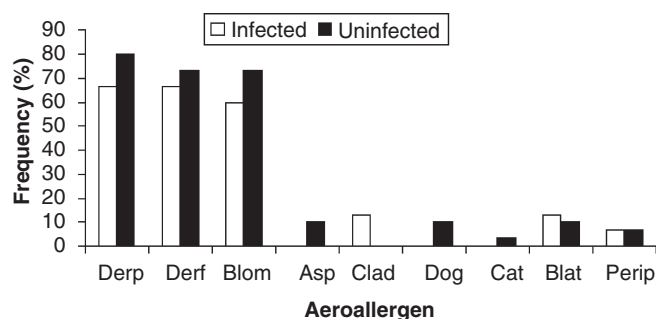


Figure 1. Frequency of positive reactions to specific aeroallergens in human T-lymphotropic virus type I-infected (asymptomatic carriers) and uninfected subjects.

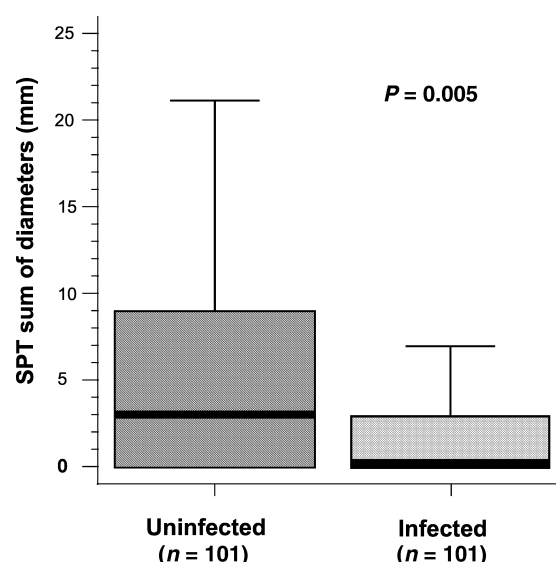


Figure 2. Sum of the diameters of the reactions to all the allergens tested in human T-lymphotropic virus type I-infected (asymptomatic carriers) and uninfected subjects, expressed as medians, 25th and 75th percentiles and range.

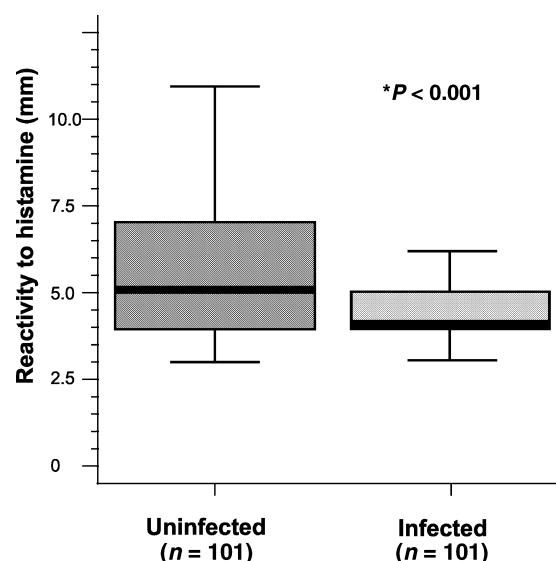


Figure 3. Skin reactivity to histamine in patients infected with human T-lymphotropic virus type I (asymptomatic carriers) and uninfected controls.

Discussion

In the present study, we showed that the frequency of atopy was lower in HTLV-I infected blood donors (asymptomatic carriers) than in an uninfected matched group. We also observed a reduction in the sum of diameters of SPT to aeroallergens. In addition to these findings, it was noteworthy a reduction in skin reactivity to histamine in HTLV-I infected individuals.

The studied patients were HTLV-I asymptomatic subjects without any chronic, consumptive or metabolic

Table 2. Association between clinical predictive variables and skin reactivity in all subjects ($n = 202$)

Factor*	β †	EP(β)‡	Odds (83% CI)	P-value§	PR (95% CI)
Serologic status HTLV-I positive	-0.44	0.64	(0.5185; 0.7989)	0.005	0.44 (0.25–0.75)
Sex (female)	0.31	1.37	(1.1047; 1.6977)	0.045	1.19 (0.99–3.23)
Family history of atopy	0.31	1.36	(1.1015; 1.6972)	0.047	1.71 (1.01–2.88)
Reactivity to histamine	0.36	1.44	(1.1423; 1.8106)	0.03	1.77 (0.97–3.22)
Sex† serologic status	-0.26	0.76	(0.6192; 0.9526)	0.09§	–
Constant	-0.28	2.74†	–	0.09	–

*Binary logistic regression for development of positive skin reactions. Conversion odds into PR (prevalence ratio) performed by means SAS® version 8.1.

†Value of each coefficient (β) in the model.

‡Wald coefficient.

§Regression test Wald *P-value.

co-morbidity. They were all candidates to voluntary blood donation and we assumed that they had no major problem with nutritional status. Variables such as age, education, income and neighborhood among participants were similar between groups. It is noteworthy that the ISAAC Phase I Study in Salvador – Brazil reported wheezing in the last 12 months and rhinitis in a frequency of 27 and 31%, respectively, among adolescents, which is exactly in the range of our healthy control group (18).

Studies have been unable to determine exactly at what age subjects become infected with the HTLV-1 so far. This lack of information is related to some features of the HTLV-1 infection, namely: (i) the majority of HTLV-1-infected subjects are asymptomatic, < 5% of the individuals progress to myelopathy (HAM/TSP); (ii) serologic tests to HTLV-I became available and mandatory in blood bank's routine in the mid-1980s. Therefore, HTLV-I asymptomatic carriers have just been unexpectedly detected by means of serologic tests in the occasion of blood donation. The HTLV-1 is transmitted via three major routes: (i) transmission from mother to child by breastfeeding; (ii) from male to female (more frequent than from female to male) by sexual contact; (iii) transmission by infected blood, either by blood transfusion or by contaminated needles among drug addicts. In Brazil, the first two routes of infection are the most frequently observed (1, 19).

Many factors can affect skin test reactions such as age, gender, race and pathologic conditions (20). The lower frequency of atopy within the group infected with HTLV-I cannot be explained by age, gender or race. It is unlikely that factors related to skin tests variability might have contributed to these differences. Skin prick tests are reproducible when performed by experienced individuals (15). No differences regarding family history of atopy was found between the groups, which have indicated that they had similar atopic backgrounds. Skin reactivity to *Der p*, *Der f* and *Blo t* were the most frequently observed in both groups. This is in accordance with previous observations made by others who have described these mites as the most frequent aeroallergens in our country (21–22).

Several studies have demonstrated an inverse correlation between infection and allergy (4–5). One of the most common explanations to this observation is the hypothesis of imbalance between Th1 and Th2 patterns of

cytokines secretion that might be deviated towards type 1 immune response by many infections (23–24). High levels of IL-2 and IFN γ have been shown in supernatants of nonstimulated lymphocyte cultures from asymptomatic HTLV-I carriers (2, 3). It is well documented that overproduction of IFN γ may inhibit many immunological functions associated to type 2 immune response (8, 9, 24, 25). The HTLV-I-infected cells also stimulate IL-18 production, which has suppressive effects selective to IL-4 and are IFN γ independent (24). Horiuchi et al. demonstrated that HTLV-I infected patients showed a significantly higher intracellular IFN γ /IL-4 ratio, lower IL-4+/IFN+ cell proportions, lower specific IgE to *Der p* and *Der f* than the noninfected control group (25). These results indicate suppression of type 2 immune response in individuals infected with HTLV-I, which might interfere with skin reactivity to allergens and are in agreement with our findings.

The SPT measures indirectly IgE mediated reactions through histamine release from mast cells in the skin. Several studies have documented that IFN γ is able to decrease IL-4 production and IgE levels, to inhibit mast cell proliferation, decrease the number of skin mast cells and suppress histamine release (8, 9, 24). Moreover, after induction of wasp venom immunotherapy, venom-induced absolute and relative histamine release was reduced and this inhibitory effect was attributed to increased production of IFN γ and IL-10 (26). Another possible explanation to our findings is that high levels of IFN γ , IL-10 and transforming growth factor (TGF) β could interfere in skin reactivity to histamine and to allergens altogether (26, 27). The TGF β inhibits IL-4 and IFN γ production by stimulated human T cells, reduces allergic inflammation through inhibition of IgE transcription and decreases mast cells proliferation (27). Interferon γ is a negative regulator of immune response type 2, inhibit histamine release and might cooperate with TGF β to modulate mast cell mediator release, in an experimental model of peritoneal mast cells (28). Moreover, Th2 cells are more affected by histamine-enhanced TGF- β 1 suppression in an H2 receptor-dependent fashion based on the cAMP pathway, which is important for the regulation of allergic immune responses (29). The immunologic events above mentioned could be present and would act down regulating immune

response type 2 and decreasing skin reactivity to allergens in a subgroup of HTLV-I infected individuals.

Intradermal injection of histamine causes vasodilatation, leakage of plasma proteins and stimulates axon reflexes within the dermis. The peptidergic nerves release neuropeptides, which induce mediators release from mast cells and cutaneous inflammation (30). Moreover, wheal and flare reactions are dependent on histamine and neuropeptides release. Skin blood vessels and nerves are responsive to histamine (30). In the present study, patients infected with HTLV-I showed lower skin reactivity to histamine than did the controls. Subjects with spinal cord injuries, multiple sclerosis or peripheral nerve abnormalities show a decrease in reactivity to skin tests (31–32). HTLV-I infection is a demyelinating condition that affects mainly the thoracic spinal cord. A low number of HTLV-I infected patients, 1–5% on the average, can progress to HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP). Progression to HAM/TSP has an insidious onset frequently lasting many years. Limbs weakness, spastic paraparesis and lumbar pain characterizes peripheral commitment associated to intestinal, urinary and sexual dysfunction in some patients (33, 34). It is noteworthy that some authors have reported peripheral neuropathy in HTLV-I infected individuals (33, 34). Aronin et al. reported that a dysfunction of peripheral nerves and the lack of neuropeptides in demyelinating disorders might potentialize the inhibitory effects of IFN γ on the skin (35). Alternatively, dysfunction of the mast cells or vascular reactivity might contribute to the diminished wheal and flare and neurogenic inflammation in neuropathic states. Thus, peripheral neuropathy might be present in some HTLV-I carriers perhaps representing the earliest nerve dysfunction in a subgroup of HTLV-I infected subjects.

In the present study, the reduced skin reactivity to allergens suggests that the type 2 immune response is suppressed in a subgroup of HTLV-I infected subjects. However, remarkable reduction in skin reactivity to histamine might have contributed as a co-factor for reduction of skin reactivity to allergens. Despite the fact that HTLV-I infected subjects were completely asymptomatic, poor reactivity to histamine highlights the hypothesis that there might exist sub-clinical peripheral neural commitment in such subjects. In order to pursue further the

question of the reduced histamine reactivity among asymptomatic HTLV-I carriers that might be attributed to an early manifestation of neuropathy we have conducted SPTs in 10 subjects with clear HTLV-I related myelopathy/tropical spastic paraparesis. The median diameter of the skin reactions was 4 mm, the same found among asymptomatic HTLV-I carriers. It suggests neuropathy is not the major factor determining skin hypo-reactivity to histamine.

In cross sectional studies, the finding of an association is no proof of causality. Moreover, one may not exclude completely the possibility that atopics are less likely to be infected with this virus. However, it seems more biologically plausible to consider the hypothesis that the inverse associations observed are related to the fact that HTLV-I infection reduces the frequency of respiratory atopic diseases, inhibits skin reactivity to allergens and skin reactivity to histamine. The mechanisms involved in such phenomena and the definitive proof of causality must be elucidated.

In conclusion, we have found a reduction in the frequency of atopic respiratory disorders and of skin reactivity to aeroallergens in HTLV-I-infected subjects (asymptomatic carriers). However, these results must be interpreted with caution, because it was also observed a pronounced reduction to skin reactivity to histamine. Suppression of skin reactivity to aeroallergens could be a local expression of complex systemic neuroimmunologic events related to suppression of histamine release and /or reactivity to histamine in the skin, in this subgroup of HTLV-I infected subjects. Further studies should be designed to determine whether in addition to suppressing skin reactivity to histamine, HTLV-I also down regulate IgE specific to aeroallergens, which mechanisms are involved, and whether T cells from atopic HTLV-I infected subjects are functionally able to elicit a response to stimulation with allergens.

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