Mudança de padrão Th1 para Th2 e atopia em indivíduos infectados por VIH? Uma revisão das evidências actuais

Th1 to Th2 pattern shift and atopy in HIV-infected individuals? A review of current evidence

Data de recepção / *Received in*: 25/08/2011
Data de aceitação / *Accepted for publication in*: 12/09/2011

Rev Port Imunoalergologia 2011; 19 (3): 135-142

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RESUMO

A infecção pelo VIH tem efeiotos profundos a nível da imunidade celular, com repercussões em muitos sistemas do organismo e processos patológicos. O número de células T CD4+ diminui com a progressão da doença ligada ao VIH, mas linfócitos B produtores de anticorpos também ficam afectados. O estado de de activação celular B anormal associado ao VIH manifesta-se por hipergamaglobulinémia e pela presença de imunocomplexos e ainticorpos circulantes. A hipergamaglobulinémia na infecção pelo VIH também inclui aumentos muito significativos dos níveis de IgE sérica total. De facto, foi demonstrado que parte deste anticorpos IgE produzidos no decurso da infecção pelo VIH são dirigidos contra fungos patogénicos comuns tais como a *candida albicans* ou contra o próprio VIH. Alterações nos padrões de citocinas produzidas por células T podem estar subjacentes a este aumento da produção de IgE específica de antigénios e colocou-se mesmo a hipótese de que possa ocorrer uma mudança da expressão de citocicas Th1 para Th2, induzida pelo VIH. Contudo, diferentes estudos mostraram resultados contraditórios em termos da associação entre padrões de citocinas Th1 e Th2 e infecção pelo VIH. Este artigo faz uma revisão dos detalhes deste contexto.

Palavras-chave: SIDA, atopia VIH, células Th2, IgE.

ABSTRACT

Infection with HIV has profound effects upon cellular immunity, with consequences for many organ systems and pathological processes. CD4+ T cell numbers decrease with progression of HIV-related disease, but antibody-producing B cells are also affected. The HIV-associated abnormally activated state of B cells manifests itself by hypergammaglobulinemia and by the presence of circulating immune complexes and autoantibodies. Hypergammaglobulinemia in HIV infection also includes highly significant increases in the levels of total serum IgE. In fact, it has also been shown that part of these IgE antibodies produced in the course of HIV infection are directed against common pathogenic fungi such as Candida albicans or against HIV itself. Changes in T cell cytokine patterns may underlie this increase in antigen-specific IgE production and it has even been hypothesized that there may an HIV-induced Th2 to Th1 cytokine expression shift. However, different studies have shown contradictory results in terms of association of Th1 and Th2 cytokine patterns and HIV infection. This article reviews the details of this context.

Key-words: *AIDS, atopy, HIV, Th2 cells, IgE.*

INTRODUCTION

Atopic allergic diseases have increased in incidence and prevalence worldwide, both in developed and developing countries, over the past 30 years[1]. Currently, about 25 to 30% of the population suffers from one of the forms of allergic disease, particularly allergic rhinitis, bronchial asthma or atopic dermatitis, even in developing nations. An estimated 6.7 million South Africans have an atopic disease, particularly those living in urban environments[2]. These result in significant morbidity as well as in socio-economic stress, and constitute an important cost to the health sector.

Allergic diseases have an underlying inflammatory component to which both type I (immediate-type) and type IV (delayed-type) mechanisms of hypersensitivity contribute[3]. Hallmarks of atopic allergic disease are an influx of eosinophils into sites of allergic inflammation, and production of high levels of allergen-specific IgE antibodies. Both of these aspects are dependent upon the third important feature of allergic diseases: a skewed clonotypic tendency in allergen-specific T cells to produce high amounts of IL-4, IL-5, IL-6, IL-9 and IL-13, and low amounts of IFN-γ and IL-2[4,5]. This is known as a Th2-type cytokine profile. This is in contrast with a Th1 type of cytokine pattern, which is rich in IFN-γ and poor in IL-4, IL-5 and IL-13[4-6] and which tends to be associated with responses to intracellular pathogens. Finally, there is a less restricted Th0 cytokine pattern, and CD4+ Th0 cells are believed to be precursors of both Th1 and Th2 type CD4+ T cells[7,8]. Although this scheme is an oversimplification of a complex process, it is consistent with a large number of observations made on specimens from the sites of disease.

South Africa has a large burden of human immunodeficiency (HIV)/acquired immunodeficiency syndrome (AIDS), with over 5.6 million people living with the disease[9], and is currently implementing the largest antiretroviral treatment (ART) programme in the world.

Infection with HIV has profound effects upon cellular immunity, with consequences for many organ systems and pathological processes. Since the T-helper cell, the primary target of HIV infection, also plays an important role in the immune dysregulation associated with the atopic state, the study of atopy during HIV infection has attracted interest over the last two decades. However, results of studies have been inconclusive and questions remain, particularly concerning the development of atopy during long-term antiretroviral therapy. This paper reviews this interaction between HIV infection and manifestations of atopy during successful ART.

SOME IMMUNOPATHOLOGICAL ASPECTS OF HIV INFECTION

CD4+ T cell numbers decrease with progression of HIV-related disease[10], but antibody-producing B cells are also affected. Cognate B cell-CD4 T cell interactions are abnormal in viraemic HIV-infected individuals[11,12] and B cells fail to adequately upregulate CD80, CD86 and DC40, following stimulation with activated T cells[11]. Since these are co-accessory receptors that are crucial for productive interactions between T and B cells, the lack of expression of these receptors contributes towards poor antigen presenting capacity by B cells and a deficient activation of T cells. Furthermore, B cells also respond poorly to CD4+ T cell help, partly due to their inability to upregulate CD25 (IL-2 receptor)[12]. This does not allow them to undergo correct activation changes induced by T cell-derived IL-2. However, in spite of the previously mentioned *in vivo* changes in B cell physiology in HIV infection, B cells are still capable of producing immunoglobulins (Igs) and, in fact, the aberrant activated state of B cells manifests itself by hypergammaglobulinemia[13] and by the presence of circulating immune complexes and autoantibodies[14].

HIGH LEVELS OF TOTAL SERUM IGE IN HIV INFECTION

Hypergammaglobulinemia in HIV infection also includes highly significant increases in the levels of total serum IgE[15-17]. Since elevated IgE levels are one of the hallmarks of atopic allergic disease, it is important to analyse whether there are also elevated levels of allergen-specific IgE antibodies in HIV-infected patients, i.e., whether there is an increased frequency of atopy in these patients. This is crucial because increased levels of total serum IgE are not only caused by allergic diseases but can be also observed in a variety of situations such as helminthic infections, certain immunodeficiencies and tumors. In fact, it has also been shown that part of these IgE antibodies produced in the course of HIV infection are directed against common pathogenic fungi such as *Candida albicans*[18] or against HIV itself[19]. Therefore, a much more reliable marker of atopy is an increase in the levels of allergen-specific IgE antibodies. On the other hand, it is known that, in allergic disease, B cells are induced to undergo isotype switching to IgE production via cognate interactions with allergen-specific CD4+ Th2-type cells[5]. Thus, en par with analysing whether there are increased levels of allergen-specific IgE antibodies, it is also crucial to ascertain whether high levels of total serum IgE in HIV infection are also associated with a preferential expression of a Th2-type profile in T cells in HIV-infected patients, although high IgE levels may not always correlate with IL-4 or IFN-γ serum levels[20].

EXPRESSION OF TH1 AND TH2 CD4+ T CELL CYTOKINE PATTERNS IN HIV INFECTION

Different studies have shown contradictory results in terms of association of Th1 and Th2 cytokine patterns and HIV infection.

Several studies have suggested that a shift from a preferential Th1-type pattern (useful for dealing with intracellular infections) to a Th2-type cytokine pattern may occur with HIV infection. One of the first studies to show this was a Dutch study in which 5 HIV-positive homosexuals, who were not on ART, and who had no allergic manifestations prior to HIV infection were compared with 2 HIV-negative controls[21]. Peripheral blood mononuclear cells (PBMC) were isolated and T cell clones (TCC) developed. TCC were stimulated with phorbol esters (PMA) and anti-CD3 monoclonal antibodies (mAb) and supernatant was collected at 24 hr (IL-2) and 72 hr (IL-4, IL-5, IL-10, IFN-γ) for analysis using a bioassay (CTLL-2) for IL-2 and ELISA for the other cytokines. Compared with TCC from HIV-negative controls, TCC isolated from HIV-infected patients consistently showed increased IL-4 production, often accompanied by increased IL-5 and decreased IFN-γ production, suggesting a switch towards Th2 clones. Furthermore,

in 2 patients from whom cells were available before and after infection with HIV, an increase in Th2 cytokines was seen after HIV-infection. In a German study, flow cytometry with staining for IL-2, IL-4, IL-10 and IFN-γ performed on mitogen-stimulated PBMC from 16 healthy donors, 18 HIV-1-infected individuals without AIDS and 14 patients with AIDS confirmed reduced percentages of IL-2 and IFN-γ (Th1 type)-producing CD4+ T cells in HIV-infected patients[22]. Furthermore, as the disease progressed both IFN-γ-producing and IL-2 expressing CD4+ T cells decreased, suggesting a relative increase in the frequency of Th2 cytokine expressing cells. In another study, which compared HIV-infected patients on ART with uninfected controls, the presence of a preferential Th2-type cytokine pattern correlated directly with HIV viraemia[23]. Serum IL-2 and IFN-γ levels were lower and IL-4 and IL-10 levels were higher in HIV-positive patients than in controls. Curiously, CD4+ T cells from low viraemia patients mainly produced IL-2 and IFN-γ, whereas in those with high viral loads, IL-4 production by CD8+ T cells was observed – Tc2 cytokine pattern. Utilizing a different approach to study the preferential expression of Th2-type cytokines in HIV infection, Chan et al[24] used antibodies against two phenotypic markers – ST2L and IL18R – and showed that CD4+ T cell lines which had a Th2-type or a Th0-type secretory pattern expressed only ST2L whereas T cell lines with a Th1-type cytokine pattern expressed only IL18R. In addition, whereas healthy volunteers had a balanced expression of ST2L+ (Th2-type) and IL-18R+ (Th1-type) CD4+T cell subtypes, HIV-infected patients had a clear predominance of the Th2--type CD4+T cell, suggesting that HIV infection is associated with a Th1 or Th0 to a Th2-type shift.

Some studies have used a broader definition of human Th2-type T cells, and have included IL-6, TNF-α or IL-10 as part of the pattern. Using this approach, a study in 81 adult HIV-positive patients (65 with total CD4+ cells count > 200/mm^3; 16 with < 200/mm^3), receiving ART, and 57 healthy controls showed that the blood of HIV-infected patients (particularly those with lower CD4+T cell counts) had decreased levels of IFN-γ and IL-12p70 and increased levels of IL-10 compared to HIV-negative controls, suggesting a possible shift from a Th1-type to a Th2 cytokine pattern[25]. Another study analysed T cell responses to HIV-derived proteins and peptides (HIV-like particles – HIV-VLPs)[26]. Blood was taken from 19 adult HIV-positive patients (11 low viraemia and higher CD4 counts; 8 high viraemia and lower CD4 counts), as well as from 4 healthy controls, and PBMC were isolated and cultured with HIV-VLPs. The average basal level of all evaluated cytokines was low, with no significant differences between HIV-negative and HIV-positive individuals. Basal IL-6 levels in both the low- and high-viraemia HIV-positive groups were significantly higher than in the control group. HIV-VLPs induced a significant increase in production of the Th2-like cytokines (IL-10, IL-6, and TNF-α) cytokines in both HIV-negative and HIV-infected samples. However, the increased production of IL-10 and TNF-α in the high-viraemia HIV-infected group was significantly lower than in the other groups; moreover, the production of Th1-associated IFN-γ was significantly increased by HIV-VLP only in the healthy group, again suggesting an impairment in production of some Th1-associated cytokines in HIV infection. Another study, which used semiquantitative RT-PCR analysis, showed that IFN-γ mRNA in unstimulated peripheral blood lymphocytes (PBL) was significantly decreased and IL-10 mRNA was significantly upregulated in HIV-infected patients with < 400 CD4+ T cells/mm^3 (n = 30) as compared to patients with > 400 CD4+ T cells/mm3 (n = 6) and normal controls (n = 16)[27]. In addition, IL-10 mRNA levels were inversely associated with IFN-γ expression. Production of IL-4 was significantly reduced in HIV-infected individuals with < 400 CD4+ T cells/mm3 as compared to the normal controls. However, the ability to produce IFN-γ by mitogen-stimulated total PBL and CD4+ purified cells was not impaired in HIV+ individuals. In another study involving 76 adult HIV-infected patients at different disease stages (31 with < 200 CD4+ T cells/mm^3; 37 homosexuals; 54 patients on ART), as well as 25 HIV-negative controls, IFN-γ release in mitogen-induced PBMC cultures was comparable in HIV-infected patients and HIV-negative controls,

whereas IL-4 production was significantly decreased in HIV-infected patients[28]. However, levels of spontaneous and mitogen-induced IL-10 production were significantly increased in HIV-infected patients, particularly in the ones with more advanced disease.

These studies which used IL-10 as a marker of a Th2 cytokine pattern have to be interpreted with caution since, as seen in the last of these studies, changes in the levels of IL-10 do not always change in parallel with IL-4, in the course of HIV infection. Furthermore, IL-10 may be produced by subsets of CD4+ T cells with regulatory properties (Tregs)[29], making interpretation of these results more problematic from a Th1/Th2 perspective.

In contrast to the above studies, other reports have failed to show a Th1 to Th2 cytokine pattern shift in HIV infection. For example, a large (n=520), longitudinal US-based study analysed mRNA and protein levels for IFN-γ, IL-2, IL-4, and TNF-α and protein levels of IL-6 in PBMC from a cohort of HIV-positive and HIV-negative adolescents[30]. Sixty-five percent were HIV-positive, in initial stages of the infection and had a median CD4+T cell count of 499 cells/mm^3. No differences in cytokines were detected between the two groups, and there was no apparent relationship between the cytokine measurements and the viral load or CD4+T cell numbers. In another study, constitutive cytokine expression was analysed in unfractionated and sorted cell populations isolated from peripheral blood and lymph nodes of HIV-infected individuals at different stages of disease[31]. Expression of IL-2 and IL-4 was barely detectable, regardless of the stage of HIV infection. CD8+ cells expressed large amounts of IFN-γ and IL-10, and the levels of these cytokines remained high but stable throughout the course of infection. Furthermore, similar patterns of cytokine expression were observed after stimulation in vitro of purified CD4+ T cell populations obtained from HIV-infected individuals at different stages of disease.

However, it is possible that the dominant shift might not be from a Th1 to a Th2 cytokine pattern, but from a Th1 to a Th0 shift. This possibility was suggested by results from a study which demonstrated reduced IFN-γ and IL-4 levels in bulk cultures of PBMC and in mitogen-induced CD4+ T cell clones from the peripheral blood of HIV-infected individuals[32]. There was a preferential reduction in clones producing IL-4 and IL-5 in the advanced phases of infection. However, enhanced proportions of CD4+ T cell clones producing both Th1-type and Th2-type cytokines (Th0 clones) were generated from either skin-infiltrating T cells that had been activated in vivo or peripheral blood T cells stimulated by antigen in vitro when cells were isolated from HIV-infected individuals. These results suggest that HIV does not induce a definite Th1 to Th2 switch, but can favor a shift to the Th0 phenotype in response to recall antigens. Furthermore, in a UK-based study, PBMC from 70 individuals with chronic progressive HIV-1 infection (clinical progressors), 10 clinical nonprogressors, and 3 immunologically discordant progressors were assessed for T cell proliferation and Th1/Th2 cytokine production[33]. Clinical progressors lacked functional HIV-1-specific T cells with proliferative and cytokine-producing capacity; clinical nonprogressors responded to a wide range of HIV-1 antigens, producing both Th1 and Th2 cytokines, suggesting that a balanced Th1/Th2 profile may correlate with successful long-term control of HIV-1 infection. Interestingly, the authors observed a rapid Th1 to Th2 shift in the response of one immunologically discordant progressor upon onset of clinical symptoms.

These divergent results regarding expression of Th2 and Th1 cytokine patterns in HIV infection are likely to be the result of differences in patient selection (age, stage of disease, co-morbidity and concurrent treatment) or methodological differences – cells used for analysis (PBMC, T cell lines, T cell clones), types of activation stimuli (PMA+ionomycin, PHA with or without anti-CD3 antibodies, HIV peptides, etc), timing of analysis, method of analysis (RT-PCR, flow cytometry, ELISA, bioassays, etc). In addition, it may be questioned whether Th1 and Th2 cytokine responses are the relevant markers of risk of atopic disease. Nevertheless, there seems to be more consistent evidence for a preferential decrease in the production of Th1-type cytokines, whether or not

this equates to a concurrent preferential expression of Th0 or Th2-type cytokine patterns with HIV infection. It also appears that shift may be influenced, in terms of timing and intensity, by the stages of the disease and immune reconstitution by the administration of ART. Regardless of its influence on atopy, a Th1/Th0 to Th2/Th0 shift may be important in terms of the pathophysiology of HIV infection since IL-4 has been shown to increase the expression of CXCR4 on CD4+ T cells[34,35], to enhance replication of HIV in these cells[35] and to inhibit the capacity of CD8+ T cells to suppress HIV replication[36]. Thus, to reverse this shift might be advantageous in the host response to HIV infection.

What do the results from the previous studies mentioned in this review tell us? Firstly, that HIV infection is a disease in which various types of dynamic changes take place at various levels, namely the immune system, throughout the various stages of the infection and during the immune reconstitution associated with ART. Figure 1 shows

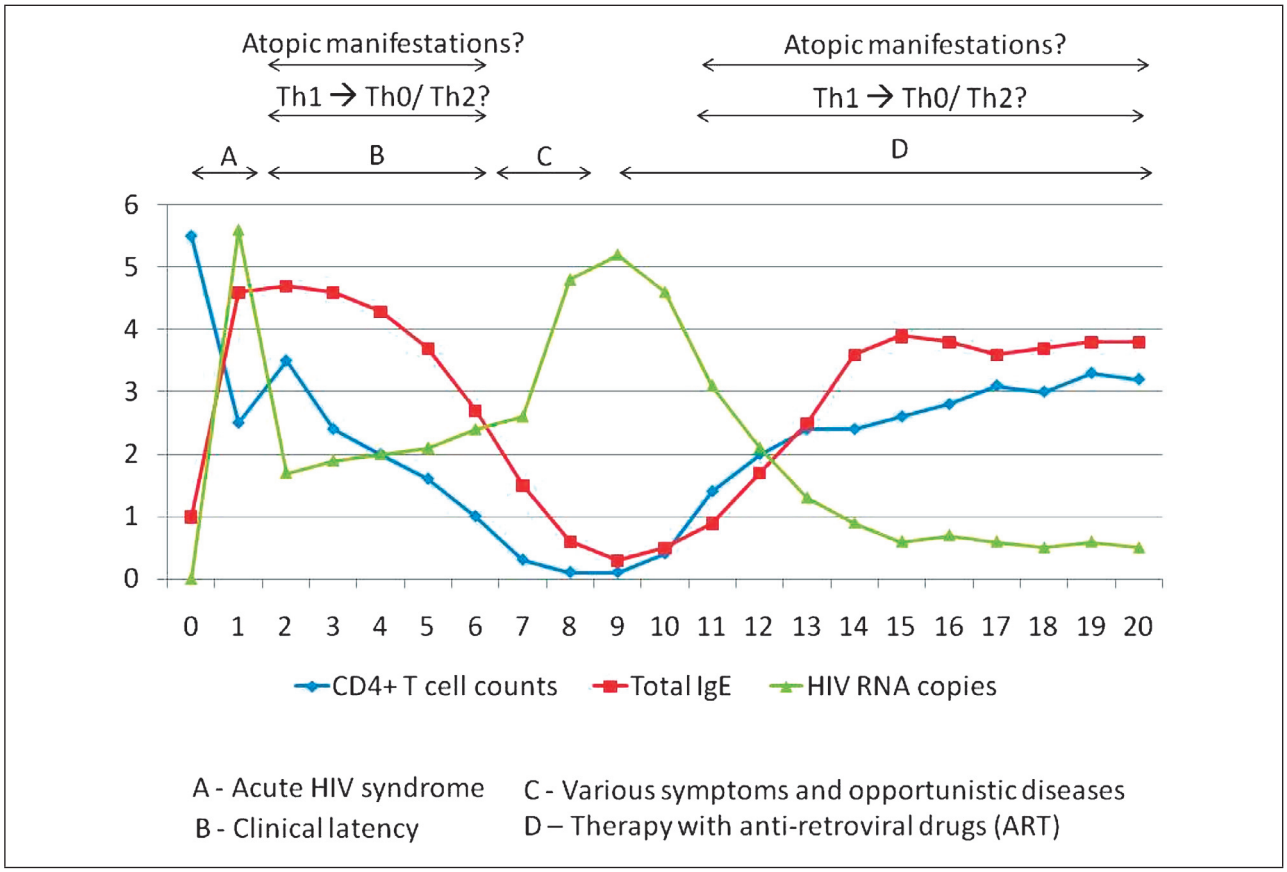

Figure 1. Time course of clinical, viral load and immunological changes in HIV infection.

(A) Initial acute HIV syndrome: flu-like symptoms are frequent, there is is seeding of the virus in lymphoid organs; (B) Period of clinical latency, during which the patients are most often aymptomatic, although there is a progressive decline in CD4+ T cell numbers, a progressive increase in viral RNA copies, and elevated levels of serum Igs, including IgE (B); (C) Period of appearance of various signs and symptoms that hallmark the development of full blown AIDS; CD4+ T cell counts are extremely low, whereas HIV RNA copies are extremely high, indicating extensive viral replication; (D) Period on ART, with stabilization of increased numbers of CD4+ T cells (immune reconstitution), and low viral loads. Th1 to Th0 or Th2 shifts may occur during periods B and D, together with increased frequencies of elevated allergen-specific IgE molecules and manifestations of allergic diseases (rhinosinusitis, bronchial asthma, eczema), although many of these manifestations may not have an atopic basis. This is an idealized graph, showing arbitrary units for both intensity (y axis) and time (x axis)

a conceptual overview of the most significant clinical and immunological changes taking place in the course of HIV infection. The second message that is important is that in order to better dissect the association between chronic diseases such as atopy-related disease and HIV infection, studies involving both cross-sectional and prospective longitudinal components should be implemented. Such studies should take place over a long period of time (4-5 years), so that the cumulative incidence of atopic sensitizations and disease may be appropriately analysed within the context of chronic, stable HIV-infection. In such cohort, cellular changes may be better analysed over time, with a more precise assessment of Th1, Th2 and other cytokine patterns and eventual time-related and/or disease--associated changes.

Funding: None
Conflict of interest disclosure: None

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Data de recepção / *Received in*: 25/08/2011
Data de aceitação / *Accepted for publication in*: 12/09/2011

Rev Port Imunoalergologia 2011; 19 (3): 135-142

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ABSTRACT

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Key-words: AIDS, Atopy, HIV, Th2 cells, IgE

RESUMO

A infecção pelo VIH tem efeiotos profundos a nível da imunidade celular, com repercussões em muitos sistemas do organismo e processos patológicos. O número de células T CD4+ diminui com a progressão da doença ligada ao VIH, mas linfócitos B produtores de anticorpos também ficam afectados. O estado de de activação celular B anormal associado ao VIH manifesta-se por hipergamaglobulinémia e pela presença de imunocomplexos e ainticorpos circulantes. A hipergamaglobulinémia na infecção pelo VIH também inclui aumentos muito significativos dos níveis de IgE sérica total. De facto, foi demonstrado que parte deste anticorpos IgE produzidos no decurso da infecção pelo VIH são dirigidos contra fungos patogénicos comuns tais como a candida albicans ou contra o próprio VIH. Alterações nos padrões de citocinas produzidas por células T podem estar subjacentes a este aumento da produção de IgE específica de antigénios e colocou-se mesmo a hipótese de que possa ocorrer uma mudança da expressão de citocicas Th1 para Th2, induzida pelo VIH. Contudo, diferentes estudos mostraram resultados contraditórios em termos da associação entre padrões de citocinas Th1 e Th2 e infecção pelo VIH. Este artigo faz uma revisão dos detalhes deste contexto.

Palavras-chave: *SIDA, atopia VIH, células Th2, IgE.*

INTRODUCTION

Atopic allergic diseases have increased in incidence and prevalence worldwide, both in developed and developing countries, over the past 30 years[1]. Currently, about 25 to 30% of the population suffers from one of the forms of allergic disease, particularly allergic rhinitis, bronchial asthma or atopic dermatitis, even in developing nations. An estimated 6.7 million South Africans have an atopic disease, particularly those living in urban environments[2]. These result in significant morbidity as well as in socio-economic stress, and constitute an important cost to the health sector.

Allergic diseases have an underlying inflammatory component to which both type I (immediate-type) and type IV (delayed-type) mechanisms of hypersensitivity contribute[3]. Hallmarks of atopic allergic disease are an influx of eosinophils into sites of allergic inflammation, and production of high levels of allergen-specific IgE antibodies. Both of these aspects are dependent upon the third important feature of allergic diseases: a skewed clonotypic tendency in allergen-specific T cells to produce high amounts of IL-4, IL-5, IL-6, IL-9 and IL-13, and low amounts of IFN-γ and IL-2[4,5]. This is known as a Th2-type cytokine profile. This is in contrast with a Th1 type of cytokine pattern, which is rich in IFN-γ and poor in IL-4, IL-5 and IL-13[4-6] and which tends to be associated with responses to intracellular pathogens. Finally, there is a less restricted Th0 cytokine pattern, and CD4+ Th0 cells are believed to be precursors of both Th1 and Th2 type CD4+ T cells[7,8]. Although this scheme is an oversimplification of a complex process, it is consistent with a large number of observations made on specimens from the sites of disease.

South Africa has a large burden of human immunodeficiency (HIV)/acquired immunodeficiency syndrome (AIDS), with over 5.6 million people living with the disease[9], and is currently implementing the largest antiretroviral treatment (ART) programme in the world.

Infection with HIV has profound effects upon cellular immunity, with consequences for many organ systems and pathological processes. Since the T-helper cell, the primary target of HIV infection, also plays an important role in the immune dysregulation associated with the atopic state, the study of atopy during HIV infection has attracted interest over the last two decades. However, results of studies have been inconclusive and questions remain, particularly concerning the development of atopy during long-term antiretroviral therapy. This paper reviews this interaction between HIV infection and manifestations of atopy during successful ART.

SOME IMMUNOPATHOLOGICAL ASPECTS OF HIV INFECTION

CD4+ T cell numbers decrease with progression of HIV-related disease[10], but antibody-producing B cells are also affected. Cognate B cell-CD4 T cell interactions are abnormal in viraemic HIV-infected individuals[11,12] and B cells fail to adequately upregulate CD80, CD86 and DC40, following stimulation with activated T cells[11]. Since these are co-accessory receptors that are crucial for productive interactions between T and B cells, the lack of expression of these receptors contributes towards poor antigen presenting capacity by B cells and a deficient activation of T cells. Furthermore, B cells also respond poorly to CD4+ T cell help, partly due to their inability to upregulate CD25 (IL-2 receptor)[12]. This does not allow them to undergo correct activation changes induced by T cell-derived IL-2. However, in spite of the previously mentioned *in vivo* changes in B cell physiology in HIV infection, B cells are still capable of producing immunoglobulins (Igs) and, in fact, the aberrant activated state of B cells manifests itself by hypergammaglobulinemia[13] and by the presence of circulating immune complexes and autoantibodies[14].

HIGH LEVELS OF TOTAL SERUM IGE IN HIV INFECTION

Hypergammaglobulinemia in HIV infection also includes highly significant increases in the levels of total serum IgE[15-17]. Since elevated IgE levels are one of the hallmarks of atopic allergic disease, it is important to analyse whether there are also elevated levels of allergen-specific IgE antibodies in HIV-infected patients, i.e., whether there is an increased frequency of atopy in these patients. This is crucial because increased levels of total serum IgE are not only caused by allergic diseases but can be also observed in a variety of situations such as helminthic infections, certain immunodeficiencies and tumors. In fact, it has also been shown that part of these IgE antibodies produced in the course of HIV infection are directed against common pathogenic fungi such as *Candida albicans*[18] or against HIV itself[19]. Therefore, a much more reliable marker of atopy is an increase in the levels of allergen-specific IgE antibodies. On the other hand, it is known that, in allergic disease, B cells are induced to undergo isotype switching to IgE production via cognate interactions with allergen-specific CD4+ Th2-type cells[5]. Thus, en par with analysing whether there are increased levels of allergen-specific IgE antibodies, it is also crucial to ascertain whether high levels of total serum IgE in HIV infection are also associated with a preferential expression of a Th2-type profile in T cells in HIV-infected patients, although high IgE levels may not always correlate with IL-4 or IFN-γ serum levels[20].

EXPRESSION OF TH1 AND TH2 CD4+ T CELL CYTOKINE PATTERNS IN HIV INFECTION

Different studies have shown contradictory results in terms of association of Th1 and Th2 cytokine patterns and HIV infection.

Several studies have suggested that a shift from a preferential Th1-type pattern (useful for dealing with intracellular infections) to a Th2-type cytokine pattern may occur with HIV infection. One of the first studies to show this was a Dutch study in which 5 HIV-positive homosexuals, who were not on ART, and who had no allergic manifestations prior to HIV infection were compared with 2 HIV-negative controls[21]. Peripheral blood mononuclear cells (PBMC) were isolated and T cell clones (TCC) developed. TCC were stimulated with phorbol esters (PMA) and anti-CD3 monoclonal antibodies (mAb) and supernatant was collected at 24 hr (IL-2) and 72 hr (IL-4, IL-5, IL-10, IFN-γ) for analysis using a bioassay (CTLL-2) for IL-2 and ELISA for the other cytokines. Compared with TCC from HIV-negative controls, TCC isolated from HIV-infected patients consistently showed increased IL-4 production, often accompanied by increased IL-5 and decreased IFN-γ production, suggesting a switch towards Th2 clones. Furthermore,

in 2 patients from whom cells were available before and after infection with HIV, an increase in Th2 cytokines was seen after HIV-infection. In a German study, flow cytometry with staining for IL-2, IL-4, IL-10 and IFN-γ performed on mitogen-stimulated PBMC from 16 healthy donors, 18 HIV-1-infected individuals without AIDS and 14 patients with AIDS confirmed reduced percentages of IL-2 and IFN-γ (Th1 type)-producing CD4+ T cells in HIV-infected patients[22]. Furthermore, as the disease progressed both IFN-γ-producing and IL-2 expressing CD4+ T cells decreased, suggesting a relative increase in the frequency of Th2 cytokine expressing cells. In another study, which compared HIV-infected patients on ART with uninfected controls, the presence of a preferential Th2-type cytokine pattern correlated directly with HIV viraemia[23]. Serum IL-2 and IFN-γ levels were lower and IL-4 and IL-10 levels were higher in HIV-positive patients than in controls. Curiously, CD4+ T cells from low viraemia patients mainly produced IL-2 and IFN-γ, whereas in those with high viral loads, IL-4 production by CD8+ T cells was observed – Tc2 cytokine pattern. Utilizing a different approach to study the preferential expression of Th2-type cytokines in HIV infection, Chan et al[24] used antibodies against two phenotypic markers – ST2L and IL18R – and showed that CD4+ T cell lines which had a Th2-type or a Th0-type secretory pattern expressed only ST2L whereas T cell lines with a Th1-type cytokine pattern expressed only IL18R. In addition, whereas healthy volunteers had a balanced expression of ST2L+ (Th2-type) and IL-18R+ (Th1-type) CD4+T cell subtypes, HIV-infected patients had a clear predominance of the Th2--type CD4+T cell, suggesting that HIV infection is associated with a Th1 or Th0 to a Th2-type shift.

Some studies have used a broader definition of human Th2-type T cells, and have included IL-6, TNF-α or IL-10 as part of the pattern. Using this approach, a study in 81 adult HIV-positive patients (65 with total CD4+ cells count > 200/mm^3; 16 with < 200/mm^3), receiving ART, and 57 healthy controls showed that the blood of HIV-infected patients (particularly those with lower CD4+T cell counts) had decreased levels of IFN-γ and IL-12p70 and increased levels of IL-10 compared to HIV-negative controls, suggesting a possible shift from a Th1-type to a Th2 cytokine pattern[25]. Another study analysed T cell responses to HIV-derived proteins and peptides (HIV-like particles – HIV-VLPs)[26]. Blood was taken from 19 adult HIV-positive patients (11 low viraemia and higher CD4 counts; 8 high viraemia and lower CD4 counts), as well as from 4 healthy controls, and PBMC were isolated and cultured with HIV-VLPs. The average basal level of all evaluated cytokines was low, with no significant differences between HIV-negative and HIV-positive individuals. Basal IL-6 levels in both the low- and high-viraemia HIV-positive groups were significantly higher than in the control group. HIV-VLPs induced a significant increase in production of the Th2-like cytokines (IL-10, IL-6, and TNF-α) cytokines in both HIV-negative and HIV-infected samples. However, the increased production of IL-10 and TNF-α in the high-viraemia HIV-infected group was significantly lower than in the other groups; moreover, the production of Th1-associated IFN-γ was significantly increased by HIV-VLP only in the healthy group, again suggesting an impairment in production of some Th1-associated cytokines in HIV infection. Another study, which used semiquantitative RT-PCR analysis, showed that IFN-γ mRNA in unstimulated peripheral blood lymphocytes (PBL) was significantly decreased and IL-10 mRNA was significantly upregulated in HIV-infected patients with < 400 CD4+ T cells/mm^3 (n = 30) as compared to patients with > 400 CD4+ T cells/mm3 (n = 6) and normal controls (n = 16)[27]. In addition, IL-10 mRNA levels were inversely associated with IFN-γ expression. Production of IL-4 was significantly reduced in HIV-infected individuals with < 400 CD4+ T cells/mm3 as compared to the normal controls. However, the ability to produce IFN-γ by mitogen-stimulated total PBL and CD4+ purified cells was not impaired in HIV+ individuals. In another study involving 76 adult HIV-infected patients at different disease stages (31 with < 200 CD4+ T cells/mm^3; 37 homosexuals; 54 patients on ART), as well as 25 HIV-negative controls, IFN-γ release in mitogen-induced PBMC cultures was comparable in HIV-infected patients and HIV-negative controls,

whereas IL-4 production was significantly decreased in HIV-infected patients[28]. However, levels of spontaneous and mitogen-induced IL-10 production were significantly increased in HIV-infected patients, particularly in the ones with more advanced disease.

These studies which used IL-10 as a marker of a Th2 cytokine pattern have to be interpreted with caution since, as seen in the last of these studies, changes in the levels of IL-10 do not always change in parallel with IL-4, in the course of HIV infection. Furthermore, IL-10 may be produced by subsets of CD4+ T cells with regulatory properties (Tregs)[29], making interpretation of these results more problematic from a Th1/Th2 perspective.

In contrast to the above studies, other reports have failed to show a Th1 to Th2 cytokine pattern shift in HIV infection. For example, a large (n=520), longitudinal US-based study analysed mRNA and protein levels for IFN-γ, IL-2, IL-4, and TNF-α and protein levels of IL-6 in PBMC from a cohort of HIV-positive and HIV-negative adolescents[30]. Sixty-five percent were HIV-positive, in initial stages of the infection and had a median CD4+T cell count of 499 cells/mm^3. No differences in cytokines were detected between the two groups, and there was no apparent relationship between the cytokine measurements and the viral load or CD4+T cell numbers. In another study, constitutive cytokine expression was analysed in unfractionated and sorted cell populations isolated from peripheral blood and lymph nodes of HIV-infected individuals at different stages of disease[31]. Expression of IL-2 and IL-4 was barely detectable, regardless of the stage of HIV infection. CD8+ cells expressed large amounts of IFN-γ and IL-10, and the levels of these cytokines remained high but stable throughout the course of infection. Furthermore, similar patterns of cytokine expression were observed after stimulation in vitro of purified CD4+ T cell populations obtained from HIV-infected individuals at different stages of disease.

However, it is possible that the dominant shift might not be from a Th1 to a Th2 cytokine pattern, but from a Th1 to a Th0 shift. This possibility was suggested by results from a study which demonstrated reduced IFN-γ and IL-4 levels in bulk cultures of PBMC and in mitogen-induced CD4+ T cell clones from the peripheral blood of HIV-infected individuals[32]. There was a preferential reduction in clones producing IL-4 and IL-5 in the advanced phases of infection. However, enhanced proportions of CD4+ T cell clones producing both Th1-type and Th2-type cytokines (Th0 clones) were generated from either skin-infiltrating T cells that had been activated in vivo or peripheral blood T cells stimulated by antigen in vitro when cells were isolated from HIV-infected individuals. These results suggest that HIV does not induce a definite Th1 to Th2 switch, but can favor a shift to the Th0 phenotype in response to recall antigens. Furthermore, in a UK-based study, PBMC from 70 individuals with chronic progressive HIV-1 infection (clinical progressors), 10 clinical nonprogressors, and 3 immunologically discordant progressors were assessed for T cell proliferation and Th1/Th2 cytokine production[33]. Clinical progressors lacked functional HIV-1-specific T cells with proliferative and cytokine-producing capacity; clinical nonprogressors responded to a wide range of HIV-1 antigens, producing both Th1 and Th2 cytokines, suggesting that a balanced Th1/Th2 profile may correlate with successful long-term control of HIV-1 infection. Interestingly, the authors observed a rapid Th1 to Th2 shift in the response of one immunologically discordant progressor upon onset of clinical symptoms.

These divergent results regarding expression of Th2 and Th1 cytokine patterns in HIV infection are likely to be the result of differences in patient selection (age, stage of disease, co-morbidity and concurrent treatment) or methodological differences – cells used for analysis (PBMC, T cell lines, T cell clones), types of activation stimuli (PMA+ionomycin, PHA with or without anti-CD3 antibodies, HIV peptides, etc), timing of analysis, method of analysis (RT-PCR, flow cytometry, ELISA, bioassays, etc). In addition, it may be questioned whether Th1 and Th2 cytokine responses are the relevant markers of risk of atopic disease. Nevertheless, there seems to be more consistent evidence for a preferential decrease in the production of Th1-type cytokines, whether or not

this equates to a concurrent preferential expression of Th0 or Th2-type cytokine patterns with HIV infection. It also appears that shift may be influenced, in terms of timing and intensity, by the stages of the disease and immune reconstitution by the administration of ART. Regardless of its influence on atopy, a Th1/Th0 to Th2/Th0 shift may be important in terms of the pathophysiology of HIV infection since IL-4 has been shown to increase the expression of CXCR4 on CD4+ T cells[34,35], to enhance replication of HIV in these cells[35] and to inhibit the capacity of CD8+ T cells to suppress HIV replication[36]. Thus, to reverse this shift might be advantageous in the host response to HIV infection.

What do the results from the previous studies mentioned in this review tell us? Firstly, that HIV infection is a disease in which various types of dynamic changes take place at various levels, namely the immune system, throughout the various stages of the infection and during the immune reconstitution associated with ART. Figure 1 shows

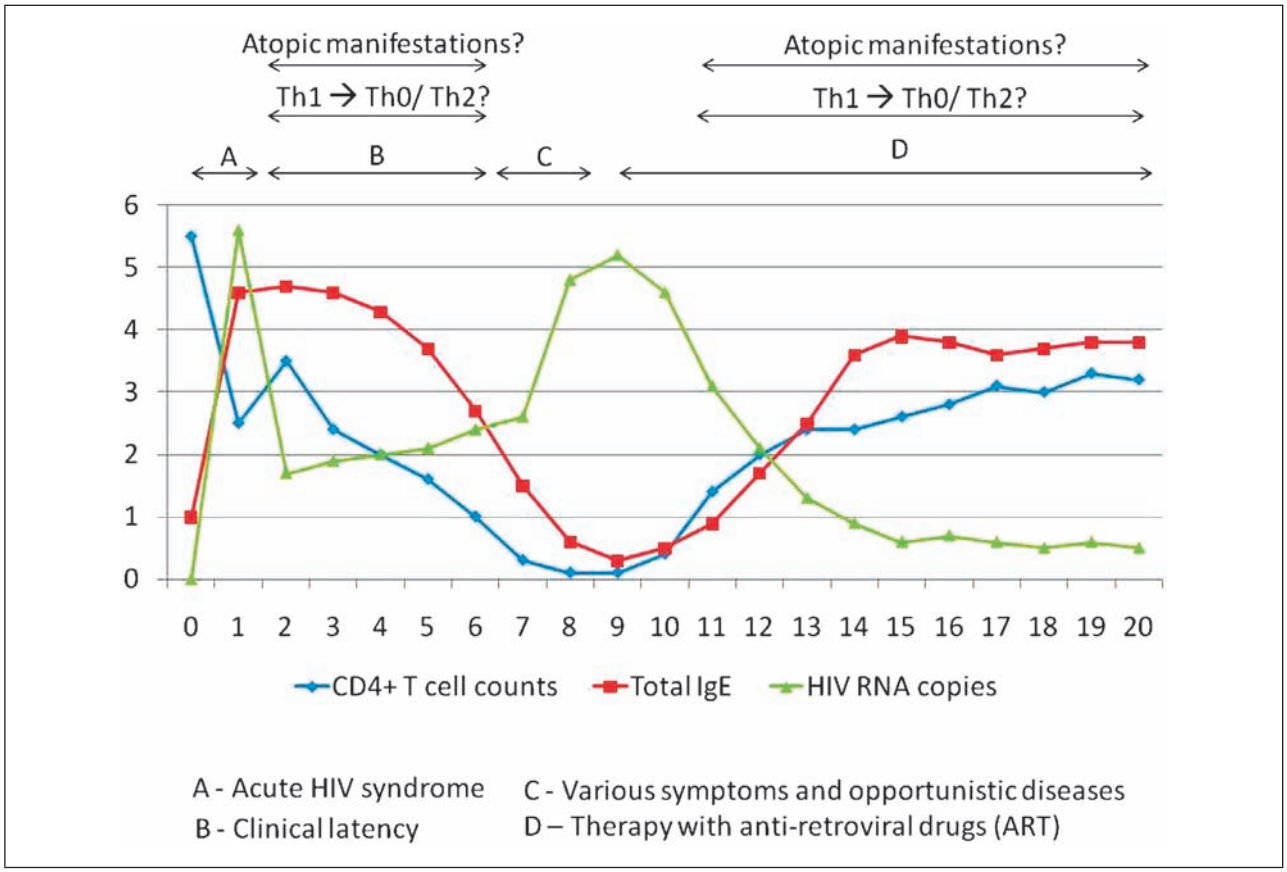

Figure 1. Time course of clinical, viral load and immunological changes in HIV infection. (A) Initial acute HIV syndrome: flu-like symptoms are frequent, there is is seeding of the virus in lymphoid organs. (B) Period of clinical latency, during which the patients are most often aymptomatic, although there is a progressive decline in CD4+ T cell numbers, a progressive increase in viral RNA copies, and elevated levels of serum Igs, including IgE (B). (C) Period of appearance of various signs and symptoms that hallmark the development of full blown AIDS; CD4+ T cell counts are extremely low, whereas HIV RNA copies are extremely high, indicating extensive viral replication. (D) Period on ART, with stabilization of increased numbers of CD4+ T cells (immune reconstitution), and low viral loads. Th1 to Th0 or Th2 shifts may occur during periods B and D, together with increased frequencies of elevated allergen-specific IgE molecules and manifestations of allergic diseases (rhinosinusitis, bronchial asthma, eczema), although many of these manifestations may not have an atopic basis. This is an idealized graph, showing arbitrary units for both intensity (y axis) and time (x axis).

a conceptual overview of the most significant clinical and immunological changes taking place in the course of HIV infection. The second message that is important is that in order to better dissect the association between chronic diseases such as atopy-related disease and HIV infection, studies involving both cross-sectional and prospective longitudinal components should be implemented. Such studies should take place over a long period of time (4-5 years), so that the cumulative incidence of atopic sensitizations and disease may be appropriately analysed within the context of chronic, stable HIV-infection. In such cohort, cellular changes may be better analysed over time, with a more precise assessment of Th1, Th2 and other cytokine patterns and eventual time-related and/or disease--associated changes.

Funding: None
Conflict of interest disclosure: None

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