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SPECIAL REPORT

The Dominant T-Helper Lymphocyte Function of HIV Infected Patients

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In HIV infection, the decrease in the number and functional activity of lymphocytes is accompanied by atopia and an increased level of total IgE and some specific IgE antibodies. This could be explained by the Th2 dominance induced by HIV replication and so a Th1-Th2 switch could have prognostic value. We investigated the characteristic T-helper phenotype dominance and its relationship to cytokine expression and IgE immune response in the early stage of asymptomatic HIV infection. In the separated lymphocytes of *i.* asymptomatic HIV positive persons; *ii.* HIV negative homosexuals; *iii.* atopic patients; and *iv.* healthy controls, expression of mRNA for IFNγ (Th1) and IL-10 (Th2) were determined by semiquantitative RT-PCR. The serum level of antibodies for HIV 1/2 and total/specific IgE were also determined. Transcription of mRNA of IFNγ and IL-10 were more pronounced in HIV positive and atopic groups than in the healthy control, without lymphocyte phenotype dominance. In HIV negative persons, however, a significant Th2 dominance was detected. There was no significant difference in the IgE level between the 4 investigated groups. In the HIV positive cases, IL-10 expression and total serum IgE do not support a switch to Th2 dominance. In the atopic group, aside from the total IgE level, down regulation of IFNγ was not observed. These results suggest a general activation of the immune system in the early stage of HIV infection. (Pathology Oncology Research Vol 3, No 1, 68–73, 1997)

Key words: HIV, Th1, Th2, RT-PCR

Itroduction

As a consequence of primary HIV infection, the loss of T helper cell function and a decline in the CD4+ T cell count can be detected. Clones of CD4+ T lymphocytes as well as CD8+ cells[3,7,17,23] produce various patterns of cytokines which determine the characteristics of the immune response. Cytokines of type I phenotype, characterised by IFN, IL-2, TNF, are responsible for delayed type hypersensitivity reaction and producing complement activating immunoglobulins. Type II clones produce cytokines (IL-4, IL-5, IL-6, IL-10, IL-13) which cause polyclonal activation of B cells resulting in increased levels of IgG1, IgA (IgM), and IgE. Earlier studies[5,15,19,21] suggested a phenotype switch from a predominant type 1 state to a predominant type 2 condition in HIV infection induced by or as a consequence of the virus. This would have had a primary role in pathogenesis and progression of the disease. Atopic-like clinical symptoms may develop and be characterised by an elevated level of total and specific IgE antibodies.[8,9,11,14,16,20,27,28] In these studies, cytokine-expression by stimulated lymphocytes from HIV-infected people was usually determined and compared to that of non-infected healthy volunteers. Other observations[1,10,12,18,25,26] seem to contradict this hypothesis or only partially support[2] the phenomenon of a Th1-Th2 switch in the course of HIV infection.

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The primary aims of our study were to *i.* analyse mRNA expression of Th1 and Th2 cytokines in non-activated lymphocytes (like "in-vivo") from asymptomatic HIV-infected persons and healthy individuals and *ii.* extend the study to two more groups, HIV negative homosexual individuals with HIV positive sexual contacts and atopic patients, which represent a broader spectrum of the population. Characteristic T helper phenotype dominance and its relationship to the cytokine expression and the IgE type immune response in the early stage of asymptomatic HIV infection were also investigated.

Materials and Methods

Study groups

Four main groups of males were investigated. Ten asymptomatic HIV-infected people without any drug administration, mean age 37 years (28-44 years); 10 HIV antibody seronegative homo/bisexual men who were sexual partners of HIV infected people, mean age 38 years (19-68 years); 10 atopic individuals with allergy symptoms, mean age 26 years (16-51 years); and 10 healthy volunteers as controls, mean age 30 years (22-53 years). Atopia have been excluded in HIV infected and healthy control groups based on former clinical and anamnestic data.

Lymphocyte separation and cell lysis

Heparinized blood samples were collected from subjects. Peripheral blood mononuclear cells (PBMC) were separated from 10-15 ml blood by Ficoll-Uromiro (Pharmacia) density gradient centrifugation, suspended in cell membrane lysing solution (guanidine isothiocyanate – mercaptoethanol, Sigma) and stored at -20°C degree until determination of IFNγ (Th1) and IL-10 (Th2) mRNA by semiquantitative RT-PCR.

Cytokine mRNA determination

After protein extraction (phenol – chloroform – isoamilalcohol, Sigma), RNAs were purified (isopropanol, guanidine isothiocyanate – β-mercaptoethanol, Sigma) according to Chomczinsky et al.[4] Reverse transcription of the RNA (1 μg) was performed in a final volume of 20 μl containing 4 μl $MgCl_2$ (25 mM), 2 μl 10× buffer II (500 mM KCl, 100 mM Tris-HCl, pH 8.3), 2 μl of each deoxynucleotide triphosphates (10 mM), 1 μl random hexamers (50 M), 1 μl RNase inhibitor (10 U/μl) and 1 μl reverse transcriptase (50 U/μl, Perkin Elmer). A DNA thermal cycler (PDR-91, BLS) was used for RT reaction with the following temperatures: 42°C, 20 min, 99°C, 10 min. The PCR reaction was performed in a final volume of 100 μl containing 10 μl RT transcript, 4 μl $MgCl_2$ (25 mM), 8 μl 10× buffer II (500 mM KCl, 100 mM Tris-HCl, pH 8.3), 1 μl each of sense and antisense primers (15 μM), 0.5 μl Taq polymerase (5 U/μl), and 75.5 μl distilled water. The assay was performed for 35 cycles of all cytokines using a DNA thermal cycler. Temperature cycling was initiated with each cycle as follows: 95°C, 30 sec, 48°C, 60 sec, 72°C, 45 sec for IFNγ primers and 95°C, 30 sec, 65°C, 30 sec, 72°C, 60 sec for IL-10 primers. Each PCR product (18 μl) was electrophoresed on a 2.5 % agarose gel and visualised under ultraviolet light after ethidium bromide staining. Cytokine mRNA expression was quantified by comparison of relative band intensity.

Analysis of sera

Sera were analysed for the presence of antibodies HIV1/HIV2 by ELISA (Organon Teknika) and for total as well as for certain specific IgE antibodies by quantitative fluoroimmunoassay (FIA, 3M). The normal level of total IgE standardized to Hungarian subjects is below 150 IU/ml.

The following specific IgE antigens were used: *Extrinsic:* Ambrosia artemisifolia (W1), Artemisia vulgaris (W6), Lolium perenne (G5), Dermatophagoides pteronyssinus (D1), Dermatophagoides farinae (D2). *Intrinsic:* egg white (F1), egg yolk (F75), milk (F2), Candida albicans (M5).

Statistical analysis

Results were evaluated by Student's t test, Spearman's rank correlation and frequency distribution (10% = 1 person). The average was calculated by positive values.

Table 1. **Phenotype dominancy based on cytokine mRNA expression of PBMCs and lymphocyte cell coun**

Patients' group	*IFNγ/IL-10 ratio**	*Dominancy*	*CD4+ cell count*	*CD8+ cell count*	*CD4/CD8 ratio (mean)*
HIV+	1.0	no	485 (335-790)	1468 (633-2454)	0.33
HIV-	0.035	IL-10 (Th2)	980 (569-1441)	610 (277-864)	1.6
atopic	0.9	no	940 (378-1509)	555 (209-1124)	1.7
healthy	4.0	IFNγ (Th1)	744 (368-1252)	739 (386-1204)	1.0

* Calculated by evaluation of relative intensity of signals of the respected cytokine's mRNA after RT-PCR.

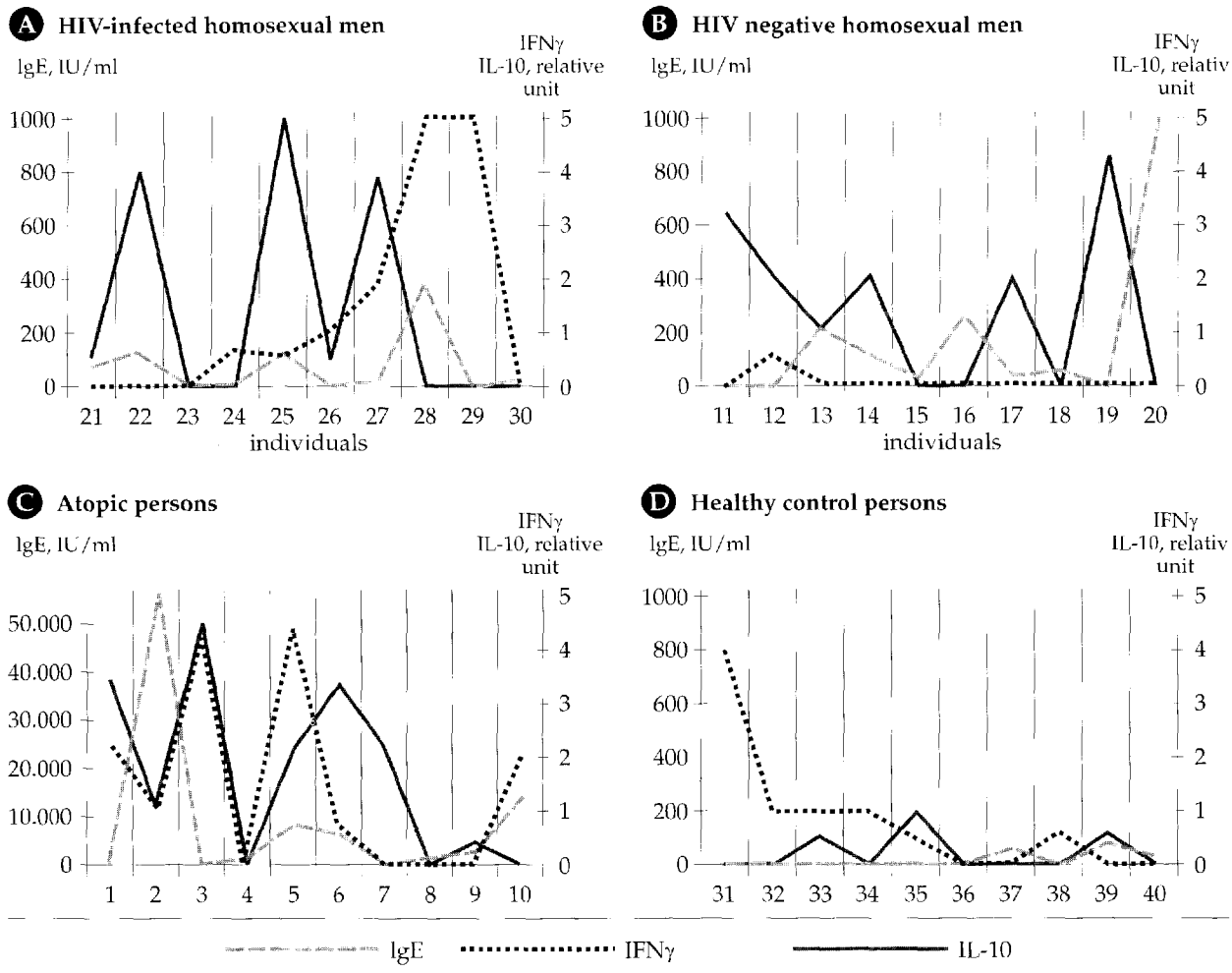

Figure 1. *Expession of IL-10 and IFNγ in PBMCs and total IgE in sera of individuals of the four study groups. Cytokine mRNA expression was determined by RT-PCR. Serum IGE was determined by FIA (see in text).*

Results

In the HIV-infected group, cytokine expression was detected in 8 cases. In 3 patients, a dominant IL-10, in 3 patients, IFNγ, and in 2 more individuals, multiple cytokine (IL-10 and IFNγ mRNA transcription was detected *(Fig 1a)*. In this group, the IFNγ/IL-10 ratio was 1.0, without a typical phenotype dominance *(Table 1)*. In the HIV negative homosexual contact group enhanced activation was measured with increased cytokines expression in 6 persons. 5 cases showed a strong IL-10, and only one showed any IFNγ mRNA transcription *(Fig 1b)*. The ratio of IFNγ/IL-10 was 0.035, with significant Th2 dominance *(Table 1)*.

In the atopic-allergic group, 8 people showed increased cytokines mRNA transcription. In 5 persons, both IL-10 and IFNγ; in 2 persons only IL-10; and in one person only IFNγ mRNA transcription was found *(Fig 1c)*. The ratio of IFNγ/IL-10 was 0.9, without the typical phenotype dominance *(Table 1)*. In the healthy control group, a decreased immune activation could be observed as compared to the other 3 groups *(Fig 1d)*. The ratio of IFNγ/IL-10 was 4.0. This demonstrates a significant Th1 dominance, which usually characterizes healthy individuals *(Table 1)*.

Table 2. **Statistical analysis of the expression of cytokine mRNA***

Groups	*IFNγ*	*IL-10*
HIV+/HIV-	p=0.05	NS
HIV+/atopic	NS	NS
HIV+/healthy	NS	NS
HIV-/atopic	NS	p=0.01
HIV-/healthy	NS	p=0.01
atopic/healthy	NS	p=0.01

* Student's t test (see Materials and methods); NS – non significant

The lowest CD4+ and the highest CD8+ cell counts were recorded in the HIV infected group. The ratio of CD4/CD8 was 0.33, however, in the other three groups, this ratio was higher than or equal to 1.0 *(Table 1.)*.

IFNγ and IL-10 values were analysed by Student's t-test to compare the different groups. In the case of IFNγ, a statistically significant difference was found between the HIV negative contact and the atopic groups (p=0.01), as well as between the HIV negative contact and HIV-infected group (p=0.05). In the atopic group and the HIV-infected group, IFNγ mRNA transcription was significantly greater than in the HIV negative contact group. In the case of IL-10, a statistically significant difference was found between healthy and atopic (p=0.01) as well as between healthy and HIV negative contact groups (p=0.01). In the atopic and HIV-infected groups, IL-10 mRNA transcription was significantly greater than in the healthy group. No cytokine dominance in the HIV infected and atopic groups was revealed by statistical analysis. This indicates rather, a general activation of immune system In the HIV-infected group, the average level of total IgE was 72 IU/ml. Except for one person, individual levels were measured in the normal range, under 150 IU/ml. In one individual, an elevated IgE value of 460 IU/ml was observed, but in this persons lymphocytes, maximal IFNγ mRNA expression was detected. We could not find any correlation between IL-10 and IgE levels *(Table 3, Fig.1a)*. In the HIV negative contact group, the average of total IgE was 192 IU/ml. In 3 persons, an elevated IgE level was measured without significant IL-10 expression *(Table 3, Fig.1b)*. In the atopic group, average total IgE was 9700 IU/ml, the highest value in the 4 groups measured. In one person, an extremely high level of IgE 58000 IU/ml, was observed. Both cytokine-levels were low, and equal to each other, in this group *(Table 3, Fig.1c)*. In the healthy group the average of total IgE was 15 IU/ml. All values were in the normal range, including low IL-10 production *(Table 3, Fig.1d)*.

Table 3. **Quantitative determination of total IgE in the sera***

Group	*Total IgE IU/ml*	*No. of persons IgE>150 IU/ml levels*	*Correlation between IgE and cytokine*
HIV+	72	1	non detectable
HIV-	192	3	non detectable
atopic	9720	10	non detectable
healthy	15	0	non detectable

* Serum total IgE was determined in a quantitative fluoroimmunoassay (see Materials and Methods)

There was no statistically significant difference in total IgE levels among the groups as analyzed by Student's t-test. There was a considerable, though, non-statistically significant difference (p=0.1) between the HIV-infected and atopic groups, contradicting our preliminary expectations *(Table 4)*.

Table 4. **Statistical analysis of total IgE serum level***

Groups	*Probability*
HIV+/HIV-	NS
HIV+/atopic	NS (P=0.10)
HIV+/healthy	NS
HIV-/atopic	NS (p=0.11)
HIV-/healthy	NS
atopic/healthy	NS (P=0.14)

* Student's test (see Materials and Methods); NS – non significant

No correlation between total IgE and CD4+ cell count could be found in the study groups, but there was a significant correlation ($p < 0.03$ – Spearman's rank correlation) between total IgE and CD8+ in the HIV negative contact group (See *Table 1 and 2*).

There was a considerable difference in specific IgE response among the groups. Based on frequency distribution, the highest frequency (%) and the highest average level of antibodies were detected in the atopic group. Healthy controls were reactive to some allergens, but the frequency and average were low (except for *L. perenne:* 5.8 IU/ml and *D. pteronyssinus* 1.2 IU/ml). *D. pteronyssinus* allergen reacted at the highest frequency in HIV positive group. A rather strong, but non-significant, correlation between IgE reactivity and IL-10 response (p=0.13 NS) was found. The results of the HIV negative contact group were similar to healthy controls, except for *D. farinae*, where the frequency distribution was 70%. There was also a statistically significant difference between D2 reactivity and IL-10 transcription (p= 0.03).

Discussion

The concept of the dichotomy of T-cell function, or a Th1-Th2 switch was described by Mossmann et al[22] in the 1980s in a mouse system. This observation has recently been extended to humans according to experiments in the early 1990's. Subsets are characterized by the difference in cytokine patterns and in effector function towards pathogens. The nature of the response to a pathogen is critical in determining disease resistance and susceptibility.[3,23] Th1 clones are involved in protection against intracellular viruses and tumors through their action on certain cytokines which induce cytotoxic and inflammatory function. Cytokines of Th2 cells are responsible for non-complement binding antibody, especially IgE, production. A third type of CD4+ cells has already been described,

termed Th0, which produces a composite Th1/Th2 cytokine pattern.[23,25] This could be a progenitor and/or a transient state of Th1 and Th2 subsets. These findings suggested that a protective effect in HIV infection should be developed by a Th1 type immune response. Some studies have reported that in HIV infection induced by or as a consequence of the virus, a Th1 to Th2 (or Th0) phenotype switch could cause immunological dysfunction which contributes to the decline of protection against HIV and other opportunistic infection. However, other reviews have contradicted these findings and the existence of a Th1 to Th2 switch in HIV infection is being a seriously debated among various research groups.[2,3,7,10,12,15,18,21,25,26]

Our recent data suggest that in the early phase of HIV infection, either Th1 or Th2 phenotype dominance can be measured because no considerable deregulation in the transcription of IFNγ (Th1) and IL-10 (Th2) is observed. In comparison to our healthy group, however, alteration of immune system function is obvious from the lack of Th1 type dominance and the considerable change in CD4+ and CD8+ cell count. Earlier studies have described elevated levels of IgE in HIV infection and allergy-like symptoms,[8,11,14,16,20,27,28] attributable to increased cytokine expression of Th2 clones which then influence B cell development.

IL-10 induces proliferation of activated B lymphocytes and secretion of immunoglobulins. In our study, correlation between IL-10 mRNA transcription and total IgE production were examined by considering the role of the regulatory effects of IL-10. However, we could not detect a significant relationship between total and specific IgE response versus IL-10 mRNA expression. A correlation between IgE and CD4+, previously demonstrated in adults with a CD4+ cell number less than 200/μl, was not observed in our study.

Specific IgE response to *D. pteronyssinus* was observed in the HIV-infected group (50%, mean 1.5 IU/ml). *D. pteronyssinus* is one of the most common aeroallergens. The majority of atopic subjects (60%) were sensitized to house dust mite (mean 164 IU/ml). In 25% of the healthy controls, a very low specific immune response without genetic disposition and symptoms (mean 1.1 IU/ml) also was measured. Results of D1 levels in HIV-infected and healthy groups are rather similar. The highest frequency of D1 specific IgE response and its rather strong but non-significant correlation to IL-10 expression may be explained by a regulated but existing Th2 type effect. For HIV negative homosexual contact individuals most studies have described an enhanced activation of immune system.[5,14] This can be explained by their sexual behaviour as the immune system of these individuals may frequently encounter infectious agents, as well as HIV antigens. In our study, a significant Th2 dominance was observed in the HIV negative contact group. This is a very important observation because, according to reports supporting a phenotype switch, a Th2 cell type dominance among high-risk individuals could indicate a relative susceptibility to HIV transmission.[5] This is supported by our recent finding that one member of the non-HIV-infected contact group acquired HIV infection during the study. According to our recent results, in HIV-infected, as well as in HIV negative contact and atopic groups, a rather strong immune response can be seen due to their immunocompromised condition in contrast to the healthy controls. The pathomechanism of deregulation, however, is quite different in these study groups.

Based on the above discussion, our results support a general immune activation, in vivo, during the asymptomatic phase of HIV infection, rather than a Th1 to Th2 phenotype switch. The possibility of a Th0-like (mixed) immune response, however, can not be excluded.

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