

No Effect of Interleukin-2 on IgE Levels Given in Addition to Antiretroviral Therapy in HIV-Infected Adults with CD4 > 300 Cells/mm³

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SUMMARY HIV-infected patients may have frequent atopy caused by an imbalance of Th1 and Th2 cytokines. The objective of the present study was to investigate whether IL-2 given in addition to antiretrovirals (ARV) would result in lower IgE levels and less allergic symptoms. Patients naïve to IL-2 (n = 28) began IL-2 plus ARV and were followed for 12 months. IgE, eosinophil and CD4 counts, HIV RNA, symptom scoring, PFT and skin prick test (SPT) were performed. It was found that the baseline median CD4 and IgE were 386.5 cells/mm³ and 63.5 IU/ml, respectively. Four patients had allergic rhinitis (AR) and 61% had a positive SPT to at least 1 antigen. At month 12, patients had higher CD4 counts ($p < 0.001$) compared to the baseline; however, there were no differences in IgE levels, allergic symptom scores or HIV RNA. The eosinophil count was higher after IL-2 administration. It was concluded that IL-2 plus ARV resulted in higher CD4 counts but had no effect on atopy.

Atopy and allergic diseases appear to be more common in HIV-infected individuals specifically in advanced HIV disease than in the general population. Elevated IgE and eosinophil levels are found in approximately 40% and 15% of HIV-infected patients, respectively.¹⁻³ HIV-infected patients are commonly affected by allergic rhinitis (AR), sinusitis, reactive airway disease (RAD) and atopic dermatitis (AD).⁴⁻⁶

The pathogenesis of the possible increased atopy and allergic conditions in HIV disease may be from an imbalance of T-helper (Th) 1 and 2 cells.⁷⁻⁹ HIV mainly infects and depletes Th1 cells resulting in a relative increase of Th2 cells. Increases in Th2 cells can lead to higher levels of Th2 cytokines spe-

cifically interleukin (IL)-4 and IL-5, which promote the production of IgE and eosinophils respectively.^{7,8}

We hypothesize that after receiving IL-2, a Th1 cytokine, patients will have lower IgE levels, lower eosinophil counts and reduced symptom scores of specific allergic diseases (AR, RAD, AD). To our knowledge, there have been no studies on the effect

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of exogenous IL-2 on atopy in HIV-infected persons.

MATERIALS AND METHODS

This study was conducted as a sub-study of the Esprit trial (an International randomized study of IL-2 plus ARV vs ARV alone on HIV disease progression in patients with CD4 > 300 cells/mm³).¹⁰ Between September 2001 and July 2002, 98 patients consented to enroll in the Esprit study and 58 of those also consented to co-enroll in this sub-study (28 and 30 patients were randomized to the IL-2 plus ARV and the ARV alone arms, respectively). In order to avoid comparing randomized subgroups prior to the completion of the present study, only the IL-2 plus ARV arm is reported here.

At each IL-2 cycle, patients received 7.5 mIU of IL-2 (Chiron Corporation, Emeryville, CA, USA) twice daily for 5 days. The cycle was repeated every 2 months for the first 6 months. After that, patients received additional cycles if the CD4 count was below 2 times the baseline or below 1,000 cells/mm³ if the baseline was more than 500 cells/mm³. The IL-2 dose was adjusted to 6 mIU or 4.5 mIU if the patient had intolerable side effects. The total follow up period was 12 months. ARV-naïve patients started ARV at baseline. The study was performed at HIV-NAT, at the anonymous clinic at the Thai Red Cross AIDS Research Center and at Chulalongkorn University Hospital. The institutional review board committee of Chulalongkorn University approved the study.

At the baseline visit (prior to IL-2 initiation) the following were performed: serum IgE level by ELISA (Cypress Diagnostics, Langdorp, Belgium), eosinophil count (Sysmex SF-3,000 cell counter, Kobe, Japan), pulmonary function test (PFT) by measurement of forced expiratory volume in 1 second (FEV₁), pre- and 15 minutes-post-bronchodilator, skin prick test (SPT) and diagnosis of AR, RAD and AD. SPT by prick method was performed on the volar surface of the forearm¹¹ for dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in 50:50 proportion), house dust, cockroach (*Blattella germanica* 1 and 2), positive control (0.01% histamine) and a diluent as negative control.¹² All antigens were manufactured by Greer laboratories, Inc. (Lenior, NC, USA). An induration of at least 3-mm above the negative control read 15 minutes after placement was considered positive. An increase of

FEV₁ by at least 12% after Salmeterol 200 mg administration was considered evidence for reversible airway obstruction. IgE, CD4, eosinophil count, HIV RNA and PFT were done at the baseline and at the final visit. SPT was performed at the baseline and repeated at the final visit only in patients who initially had a positive SPT to at least 1 antigen.

The criteria for the diagnosis of allergic diseases were as follows. AR: at least 2 symptoms and/or signs (symptoms: sneezing, rhinorrhea, nasal pruritus, nasal congestion, signs: nasal obstruction associated with mouth breathing, pale and enlarged nasal turbinates, clear nasal secretions, non-purulent postnasal drip, allergic shiners, allergic salute) and at least one positive SPT to either house dust, dust mite or cockroach antigen. RAD: at least 1 symptom and/or sign (symptoms: cough, night cough, dyspnea, shortness of breath, sputum production, signs: coughing, wheezing) and reversible airway obstruction by PFT. AD: at least 2 symptoms and/or signs (symptoms: skin pruritus, scaly rash with flexural involvement, signs: papules, minute vesicles, patches, with crusting and lichenification and flexural involvement).

At follow up, symptom scoring based on patient's subjective comparison of his/her symptoms with the visit 4 months previously was made. Symptom scores were as follows: -3 = much worse, -2 = moderately worse, -1 = slightly worse, 0 = no change, +1 = slightly better, +2 = moderately better, +3 = much better

The comparisons between the baseline and month 12 data expressed in median and proportion were done by Wilcoxon signed ranks test and McNemar test respectively. The change of serum IgE levels and CD4 count was analyzed by using Mann-Whitney U test while the effect of the IL-2 treatment and covariate factors on the outcome of the IgE response (IgE < 100 IU/ml) used a univariate logistic regression model. The overall level of statistical significance was set at $\alpha = 0.05$. All statistical analyses were performed using Statistical Product and Service Solutions (SPSS) for Windows, version 9.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Baseline characteristics

The patients were mainly in their mid-30s with a high median CD4 cell count of above 350

cells/mm³ and a low median HIV RNA of 1.7 log (Table 1). Sixty-eight percent and 57% had HIV RNA less than 400 and 50 copies/ml, respectively. Fifty percent were ARV-experienced of whom 86% were on HAART. Importantly, there were no patients with a CD4 count of less than 200 cells/mm³, a level considered severely immunodeficient.

The diagnosis of AR based on our criteria was made in 4 of 28 patients (14%). None had RAD or AD although one patient had reversible airway obstruction without symptoms. The median IgE level was normal, 63.5 IU/ml. Only 4 (14%) had IgE levels above 100 IU/ml, a level considered high. Men and women had similar IgE levels. Patients with diagnosed allergic diseases had a slightly higher median IgE level compared to those without (87 [IQR 52-500] vs 61.5 [53.8-65.8] IU/ml, $p = 0.355$). The eosinophil count was also normal with a median of 190 (IQR 100 - 313) cells/mm³. Only two patients (7.7%) had high eosinophil levels of more than 660 cells/mm³.

Table 1 Baseline characteristics of the patients

Characteristics	Data
Number of patients (%)	28 (48)
Mean age (SD) years	38.8 (11.2)
Gender M:F n (%)	12:16 (43:57)
Median CD4 (IQR) cells/mm ³	387 (322-552)
Median HIV RNA (IQR) log ₁₀ copies/ml	1.7 (1.7-3.0)
n (%) HIV RNA < 400 copies/ml	19 (67.9)
n (%) HIV RNA < 50 copies/ml	16 (57.1)
ARV naïve: experienced n (%)	14 : 14 (50:50)
Diagnosis n (%)	
Allergic rhinitis	4 (14)
Reactive airway disease	0 ¹
Atopic dermatitis	0
Median IgE level (IQR) IU/ml	63.5 (53.8-66)
Median absolute eosinophil count (IQR) cells/mm ³	190 (100-313)
Positive SPT n (%)	
House dust	13 (46.4)
Dust mites	15 (53.6)
Cockroach	10 (35.7)
More than one antigen	16 (57.1)

¹One patient had reversible airway obstruction without symptoms.

Sixty-one percent had a positive SPT to one or more of the following antigens: house dust, dust mite and cockroach. More positive SPT responses were elicited by house dust (46.4%, median 5.0 mm wheal) and dust mite (53.6%, median 6.0 mm wheal) than by cockroach (35.7%, median 4.8 mm wheal). The patients with positive SPT had similar IgE levels (median 65 [IQR 53.5-91.5] IU/ml) to the patients without positive SPT (median 60 [IQR 52-65] IU/ml). IgE levels above 100 IU/ml did not correlate with positive SPTs ($p = 0.132$).

Effect of IL-2 on IgE levels and allergic diseases

At month 12, all patients completed the study. The log₁₀ IgE levels were similar at baseline and at month 12 (Fig. 1). However, the median eosinophil count was higher at month 12 (190 [IQR 100-312.5] vs 275 [IQR 200-462.5] cells/mm³; $p = 0.001$) (Fig. 2). The patient with reversible airway obstruction at baseline had a normal PFT at the final visit. Loss of positive SPT to at least one antigen was seen in 47% of patients. There was a trend towards decreasing wheal size to dust mite ($n = 10$) and cockroach antigens ($n = 8$) in patients who had a positive SPT to that particular antigen at baseline.

We evaluated whether IL-2 might decrease IgE levels in subgroups of patients considered to be either atopic or having inadequately controlled HIV disease at baseline. Univariate analysis did not show a significant change in IgE levels between baseline and month 12 in subgroups of patients with the following baseline characteristics: IgE > 100 IU/ml, eosinophil

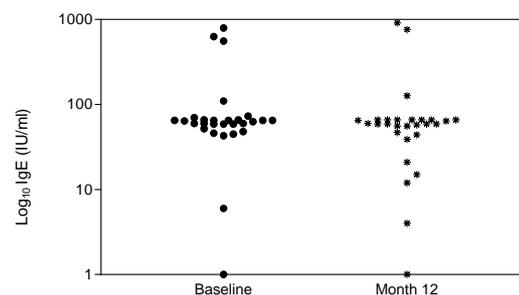


Fig. 1 Comparison of log₁₀ IgE levels at baseline and at month 12.

count > 660 cells/mm³, HIV RNA > 10,000 copies/ml, ARV-naïve, diagnosed allergic diseases, male gender and positive SPT.

Effect of IL-2 on CD4 counts and HIV RNA

At month 12, 92% were on HAART. ARV was started in naïve patients at baseline, and in some ARV-experienced patients, ARV regimens were modified according to the physician's discretion. Patients received a median of four cycles (3 to 5) of IL-2 within this period. Fig. 3 shows higher median CD4 counts after IL-2 with a median change in CD4 count from baseline of 252 cells/mm³ ($p < 0.001$). There was no change in HIV RNA.

The ARV-naïve patients had a median rise of 329 (IQR 44.8 to 579.5) CD4 cells while the median CD4 count was increased by 206 (IQR 45 to 392.3) cells in the ARV-experienced group, ($p = 0.482$).

DISCUSSION

Previous reports have shown a higher incidence of elevated IgE levels in persons with HIV infection compared to those without, with an incidence of 31 to 60% of HIV-infected having high IgE.^{1,4,8,13,14} There also appears to be a correlation between high IgE levels and advanced HIV disease status. In one study, there was a significant association between persistent IgE elevation and a decline in CD4 counts.⁸ The definition of high IgE levels varies between studies from above 45 to 150 IU/ml. We took the middle ground and chose an IgE level above 100 IU/ml to be high in our study, which was also in accordance with the IgE kit manufacturer's information. In our study, the median IgE level was 63.5 IU/ml with 14% having high IgE levels.

Published reports of healthy Thai adults showed a mean total IgE level in the serum of 24.7 IU/ml,¹⁵ with higher IgE levels in subgroups with positive SPT (97.6 IU/ml),¹⁵ airway hyper-reactivity (257.0 IU/ml) and presence of allergic symptoms (125.9 IU/ml).¹⁶ Overall, our patients had a high median IgE level. But there was no correlation between high IgE levels and positive SPT, which suggests that some of the positive SPT may have been an irritant effect. Patients with diagnosed allergic diseases had a slightly higher IgE levels than those

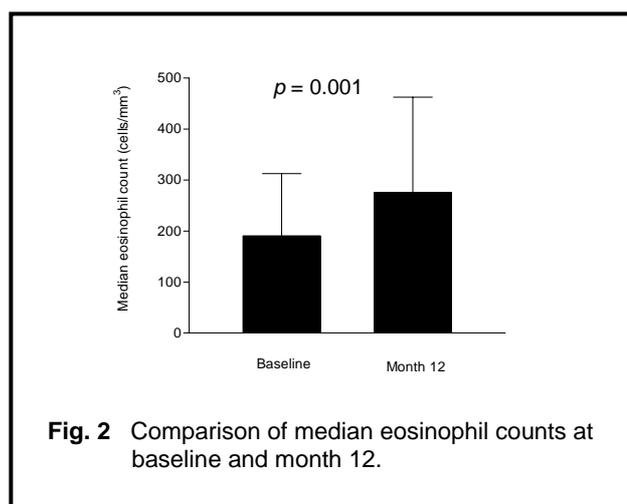


Fig. 2 Comparison of median eosinophil counts at baseline and month 12.

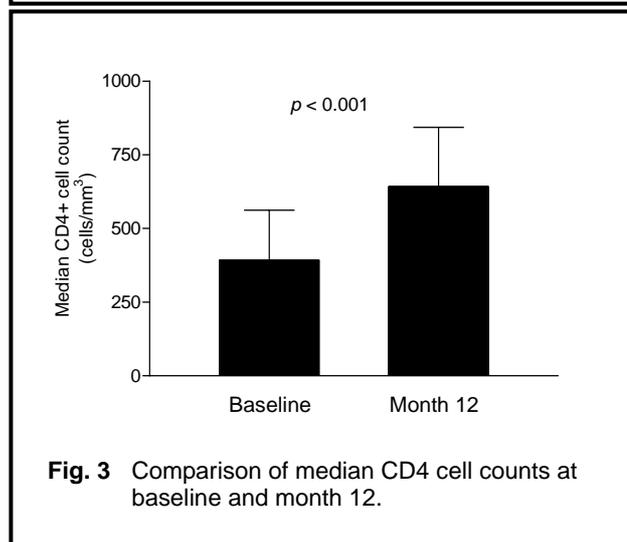


Fig. 3 Comparison of median CD4 cell counts at baseline and month 12.

without. Women were reported to have lower IgE levels than men.² Our study involved more women than men and we did not see a difference between IgE levels of the two genders.

The incidence of AR in our HIV-infected patient population with high CD4 counts was similar to a published cohort of HIV-uninfected Thai adults.¹⁷ We were not able to assess the differences in incidences of RAD and AD with the uninfected population, as there were no patients with RAD or AD in our study. Nevertheless, our study showed that HIV disease if well controlled poses no additional risk for specific allergic diseases.

HIV-infected patients may be prone to having a positive SPT to exposed antigens. Small *et al.*⁶ reported a positive SPT to at least 2 antigens in 72% of

HIV- positive patients with sinusitis. In a study of 100 non-HIV-infected Thais with clinical presentation of AR, 50% had a positive SPT to at least one of the indoor allergens.¹⁸ House dust, dust mite and cockroach antigens were chosen for SPT in our study because they represent the antigens to which Thais are most sensitized.¹⁸ About half of our patients had a positive SPT. It is, however, difficult to compare the incidence of positive SPT in our patients to others as SPT was done with different antigens at different times.

The peripheral blood eosinophil count can be elevated in HIV and allergic diseases; however, it is non-specific.¹⁹ Our patients had normal median eosinophil counts at baseline and at month 12 although the eosinophil count was higher at month 12. Interleukin-2 can stimulate eosinophil proliferation and function.²⁰ Eosinophilia is seen in patients receiving IL-2 for non-Hodgkin's lymphoma.²¹

With regards to the exogenous interleukin-2 stimulating T cell proliferation, our group has shown that exogenous IL-2 given in addition to ARV significantly increases the CD4 cell count compared to ARV alone without affecting HIV RNA.²² Similarly, in this cohort, after a median of four IL-2 cycles, patients had a significant rise in their CD4 counts of 252 cells/mm³. The CD4 rise was similar in both groups of patients, ARV-naïve and ARV-experienced at baseline, suggesting an IL-2 effect.

The fact that we did not see an effect of IL-2 on IgE levels and allergic symptoms is likely due to our patients having well controlled HIV disease, high CD4 counts and low HIV RNA. Moreover, no one had a CD4 count below 200 cells/mm³ - a level likely to have a Th1 and Th2 imbalance. Another important limiting factor was our sample size. The increase in eosinophil counts was likely due to IL-2.

In summary, after one-year of IL-2 plus ARV, patients had higher CD4 counts but similar IgE levels compared to the baseline. Successful ARV treatment, a high baseline CD4 count, a low incidence of allergic diseases and low baseline IgE levels may have contributed to our inability to show an effect by IL-2. Our patients with HIV infection had a similar incidence of allergic rhinitis and higher median IgE levels compared to reported cohorts of

HIV-uninfected Thais. Whether IL-2 in conjunction with ARV therapy has more effect on atopic diseases in an advanced HIV-infected population requires further investigation.

ACKNOWLEDGEMENTS

The authors are grateful to the patients who participated in this study. We thank Ms. Laongdown Borisut for performing PFT, and the HIV-NAT staff for helping with the study especially Ms. Bulan Seengam, Ms. Thidarat Jupimai, Ms. Narumon Subsri, Ms. Tippawan Ruanruang and Ms. Siri-orn Paksin. We also thank the Esprit committee for reviewing this manuscript.

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