

Anisakis antigen interferes in IL-4 analysis

M. Rodero, C. Cuéllar*

Key words: *Anisakis simplex*; antigens; cross-reactivity; cytokines; ELISA; IL-4.

● *ANISAKIS simplex* antigens enhance Th2 responses in animals and man (1–3). We conducted experiments to evaluate *in vitro* antigen-specific lymphocyte proliferation responses, as well as IL-4 production, with spleen cells from

BABL/c mice were injected intramuscularly with 1 mg of crude *A. simplex* antigen in Freund's complete adjuvant. The animals were killed 10 days after the immunization, and spleen cells were placed in flat-bottomed, 96-well microtiter plates (COSTAR). Samples containing 2.5×10^5

***A. simplex*-derived molecules share cross-reactive epitopes with the host cytokine IL-4.**

cells per well were plated with 1, 2, 4, or 8 μg of antigen in 100 μl RPMI 1640 medium with 10% FCS. Control cultures were cells stimulated with concanavalin A (Con A; SIGMA) at 2.5 $\mu\text{g}/\text{ml}$ or medium alone. The plates were incubated at 37°C in a humidified atmosphere of CO_2 in air for 96 h, and assayed for proliferation by colorimetric MTT (data not shown), or for IL-4 determination in the culture supernatants. IL-4 levels were detected by a commercial ELISA system according to the manufacturer's specifications (mouse IL-4, Cytoscreen[®] immunoassay kit, BioSource International, Inc., Camarillo, CA, USA). Because of the use of a biotinylated second antibody in the ELISA kit employed, and the high distribution of biotinyl enzymes in *Anisakis* (4), we tested the IL-4 levels of supernatants obtained at time 0, after the addition of the highest concentrations of antigenic extract (4 or 8 $\mu\text{g}/\text{well}$). We obtained reactivity levels (162.4 and 274.3 pg of IL-4/ml, with 4 and 8 μg of *A. simplex* antigen/well, respectively) much higher than those obtained after 3 days of culture (0.4, 65.1, 3.6, 7.5, 12.6, and 29.5 pg of IL-4/ml, with medium; Con A; or 1, 2, 4,

and 8 μg of *A. simplex* antigen/well, respectively).

For confirmation of this finding, larval *A. simplex* extract was diluted from 5 mg/ml to 3.9 $\mu\text{g}/\text{ml}$ and tested in duplicate by means of the mouse IL-4, Cytoscreen immunoassay kit with 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9, and 1.9 μg of antigenic protein per well. The concentrations of mouse IL-4 in pg/ml reached values from 297.8 to 48.5 pg/ml, as shown in Fig. 1, that were proportional to the dose of antigen used. IL-4 levels were also observed in the *A. simplex* antigen when tested by a system for human IL-4 determination (IL-4 endogen interleukin-4 ELISA, Endogen, Inc., Woburn, MA, USA) (data not shown).

Work is underway to determine whether these parasite-derived molecules that share cross-reactive epitopes with the host cytokine IL-4 are merely artifacts or do have biologic significance.

*Department of Parasitology
Faculty of Pharmacy
Complutense University
28040 Madrid
Spain
Fax: +0034913941815
E-mail: cuellarh@eucmax.sim.ucm.es

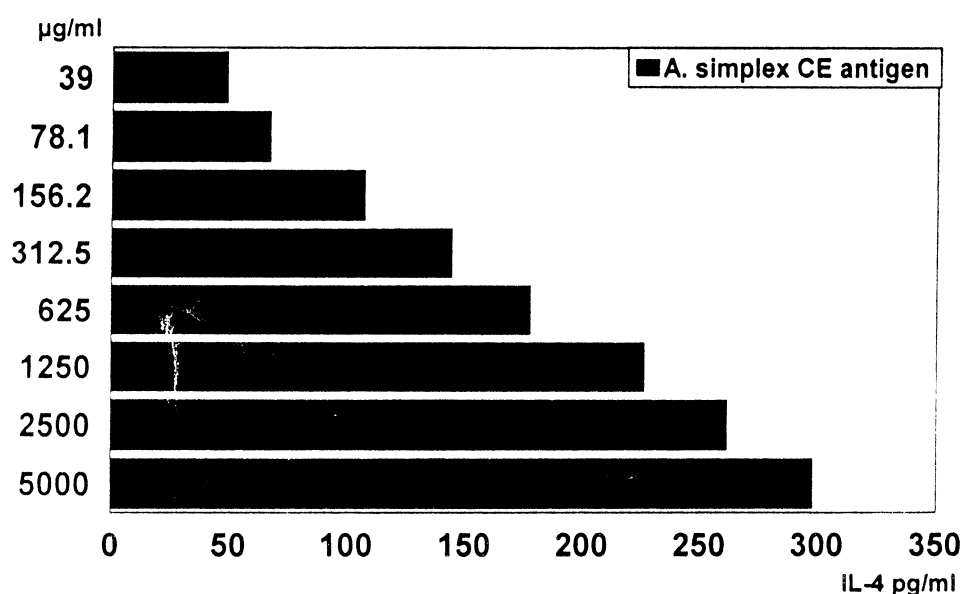


Figure 1. Values of pg/ml of IL-4 when tested with *A. simplex* antigen by mouse IL-4, Cytoscreen immunoassay kit.

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Increased END/EPX in ongoing asthma

J. Morioka, M. Kurosawa*, H. Inamura, R. Nakagami, Y. Mizushima, Y. Omura, M. Shibata, J. Chihara

Key words: bronchial asthma; EDN; ELISA.

● EOSINOPHIL-derived neurotoxin (EDN), also called eosinophil protein X (EPX), is the only one of the four basic eosinophil granule proteins that can be accurately measured in urine. A high level of urinary EDN excretion was found in patients with Churg-Strauss syndrome (1, 2), and urine EDN has been suggested as a valid alternative to the measurement of serum eosinophil proteins in child-hood asthma (3). However, there has been no study of the changes of blood

Blood and urine levels were increased when symptoms were active.

and urine EDN levels in bronchial asthma with and without active symptoms. Very recently, we developed an efficient ELISA method for measuring specifically human blood and urine EDN with a minimum detection limit of less than 0.62 ng/ml, and demonstrated that EDN concentrations in blood and urine from asymptomatic asthmatic patients were significantly raised compared with healthy control subjects (4). This report shows for the first time that blood and urinary concentrations of EDN were increased in bronchial asthmatic patients with active symptoms.

Seven bronchial asthmatic patients (two males and five females, aged 14–71 years) underwent the test. The diagnosis of bronchial asthma was established according to internationally accepted criteria (5), after assessing reversible airway obstruction and/or nonspecific bronchial hyperresponsiveness to methacholine. The mean screening FEV₁ measured when the subjects were free of symptoms was normal ($92.0 \pm 8.8\%$ of predicted), and bronchial challenge test with methacholine showed a PD₂₀ FEV₁ of less than 1 mg in all subjects. Written informed consent was obtained from each subject before the study commenced. No patients

were given prednisolone, and they were instructed to take only regular medications throughout the test. Blood and urine were obtained twice from each patient as described below. All subjects were free of symptoms for the 2 weeks preceding the initial measurements of blood and urine EDN levels and had no detectable symptoms at the time of the initial study. In addition to this test, they came to the hospital for the second measurements when they had the first active asthmatic symptoms spontaneously after the initial study, such as wheezing and breathlessness on moderate exercise (FEV₁ was $58.6 \pm 7.5\%$ [mean $\pm$ SEM] of predicted). Blood and urine levels of EDN were measured by an efficient ELISA method for EDN (4). Urinary creatinine (Cr) levels were determined with an L-type creatinine-F assay system, and urinary EDN concentrations are expressed as μg per millimole of Cr ($\mu\text{g}/\text{mmolCr}$) to correct for urine volume. The Wilcoxon signed-rank test was used to compare paired data.

Mean values $\pm$ SEM of serum, plasma, and urine EDN from the patients when they were free of symptoms were 72.2 ± 19.6 ng/ml, 44.4 ± 14.2 ng/ml, and 76.8 ± 20.7

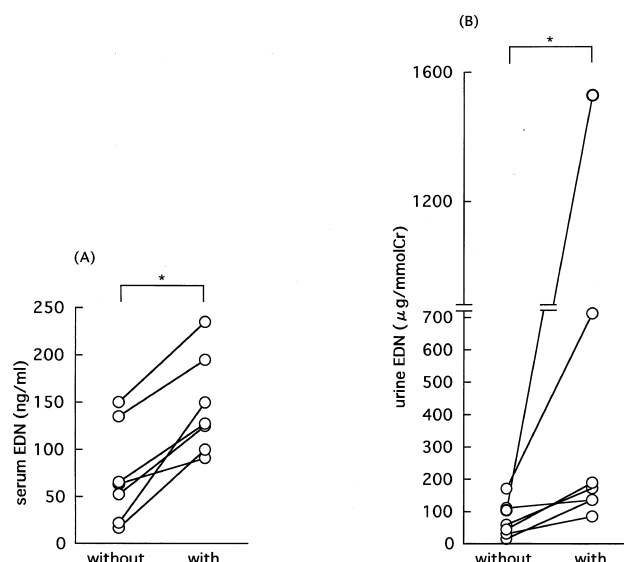


Figure 1. Comparison of EDN levels in serum (A) or urine (B) among seven bronchial asthmatic patients between times when they were without symptoms and with active asthmatic symptoms. *Significant difference at $P < 0.02$.