FOLIA HISTOCHEMICA
ET CYTOBIOLOGICA
Vol. 47, No. 1, 2009
pp. 93-97

IFN-gamma, IL-5, IL-6 and IgE in patients infected with *Giardia intestinalis*

Joanna Matowicka-Karna, Violetta Dymicka-Piekarska, Halina Kemona

Department of Clinical Laboratory Diagnostics, Medical University of Białystok, Białystok, Poland

Abstract: The immune system, its cellular and humoral response, is engaged by the host organism to fight against parasitic infections. The study group consisted of 90 patients (58 women and 32 men), aged 18-72 years, infected with *G. intestinalis*. The diagnosis was established based on laboratory investigations (stool examination, choloscopy, GSA-65). Blood for analysis was collected before (G1), and 2 weeks (G2) and 2 months (G3) after antiparasitic treatment. Control group consisted of 40 healthy subjects (22 women and 18 men), aged 20-45 years. The concentrations of IgE were assayed using a set of VIDAS (bioMerieux) and the concentrations of IL-5, IL-6, IFN-γ were determined using a set of Quantikine human (R&D Systems). It was revealed that in *giardiosis* the concentrations of IgE and IL-5 in blood serum were twice as high, the concentration of IL-6 was two and a half times higher and the concentration of IFN-γ was almost four times higher as compared to healthy controls.

Key words: IgE, IL-5, IL-6, IFN-γ , *giardiosis*

Introduction

Parasitic infections in the body are the source of foreign antigens and exotoxins that trigger local or systemic inflammatory processes [1,2]. Antibody-dependent cell cytotoxicity, in which eosinophils act as the effector cells, is the major antiparasitic defence mechanism. A few mechanisms are involved in this antibody-dependent antiparasitic immunity, causing blockade of the receptor on the parasite's surface, inducing parasite damage through activation of the complement system, or increasing IgE production. Jimenez *et al.* [3], who studied the effect of treating mice with excretory and secretory G. intestinalis antigens, observed elevated levels of total IgE as well as systemic and local stimulation of IgA secretion, both due to IL-6 production. Earlier, Zhou *et al.* [4] found that IgA plays a crucial role in G. intestinalis infection, since IL-6 regulates its production [5]. IL-6, a factor that regulates defense mechanisms, is involved in the immune response, inflammatory reaction and haematopoiesis. It stimulates the synthesis of acute phase proteins in the liver and activates progenitor cells of bone marrow, differentiation of megakaryocytes and production of blood platelets. Incubation of blood platelets with IL-6 conditions the increase in platelet cytotoxicity to *Schistosoma mansoni* larvae [6]. In mice with giardiosis the level of IL-6 was elevated and IFN-γ production by CD4+ lymphocytes was increased [7].

IL-5 produced by Th lymphocytes (mainly Th2) is involved in the induction of the increase and differentiation of B lymphocytes, cytotoxic T lymphocytes, basophils and eosinophils. IL-5 stimulates proliferation and differentiation of their precursors, their degranulation and production of oxygen reactive compounds. It exerts a chemotactic effect on eosinophils and induces eosinophilia [8].

Intracellular bacterial and parasitic pathogens, *e.g. Toxoplasma gondii*, strongly stimulate macrophages to produce IL-12 [9]. IL-12 stimulation enhances IFN-γ production by Th1 lymphocytes and this is a major mechanism in anti-infectious immune response directed against protozoa (*T. gondii*). *in vitro*, IL-12 increases apoptosis of eosinophils. The proinflammatory action of IL-12 may be the result of its stimulatory effect on the production of inflammatory proteins MIP-1α and MIP-1β [10]. Concomitant administration of IL-5 and IL-12 eliminates eosinophilic apoptosis, thus suggesting that these two interleukins have antagonistic effects on each other [11].

Correspondence: J. Matowicka-Karna, Department of Clinical Laboratory Diagnostics, Medical University of Bialystok, Waszyngtona Str. 15A15-269 Białystok, Poland;
tel.: (+4885) 7468584, fax.: (+4885) 7468584;
e-mail: matowic@amb.edu.pl

Folia Histochem Cytobiol. 2009:47(1): 93 (93-97)
doi: 10.2478/v10042-009-0013-3

According to some authors, in response to *Giardia* antigen T lymphocytes release lymphokines, and mast cells release cell mediators, prostaglandins and kinine protease. Sometimes *giardiosis* is asymptomatic (carrier state). High invasiveness of *G. intestinalis* was noted in cases with IgG and IgA deficiency [2,12].

The study objective was to find out whether *G. intestinalis* infection affects immunization parameters, and whether the antiparasitic treatment applied alters these parameters?

Materials and methods

The study involved 90 patients (58 women and 32 men, aged 18-72 years) infected with *Giardia intestinalis*, who were hospitalized in the Observation-Infectious Diseases Department or admitted in the Outpatient Department. *Giardiosis* was diagnosed based on the clinical picture, parasitological examination of faeces, enzyme immunoassay of faeces for detection of specific protein GSA-65 and microscopic examination of duodenal contents. The patients were treated with Metronidazol (c. 10 days), Tinidazole (2 days), cholagogues (Cholestil, Cholamide) and bioregulators of physiological intestinal flora (Lakcid). Blood for analysis was collected prior to treatment (G1), two weeks after antiparasitic treatment (group G2 – 60 patients – 41 women and 19 men) and two months after termination of treatment (group G3 – 43 patients – 33 women and 10 men).

Control group (C) consisted of 40 healthy subjects (aged 20-45 years), including 22 women and 18 men. All the parameters examined in study group and control group, with regard to age and gender, were subjected to statistical analysis. According to the *Guidelines for Good Clinical Practice* all the patients gave consent for participation in the study. Venous blood collected for clot was the material for analysis.

Total IgE level was determined in 100 µl serum using ELFA, on an immunoserological analyzer VIDAS with a set of reagents (bioMerieux). The set contains conjugate (mouse anti-IgE monoclonal antibody conjugated with alkaline phosphatase), rinsing buffer (sodium phosphate, pH=7.4) and substrate (4-methyl umbelliferyl phosphate 0.6 mmol/l). Human IgE combined with horse serum and metakresol (1.4 g/l) acts as a control sample and a calibrator. The sensitivity threshold of the method is 0.5 KIU/l.

Interleukin 5 (IL-5) was determined in 200 µl serum using ELISA method with a set of Quantikine human IL-5 (R&D). Microplatelets with wells containing mouse anti-IL-5 monoclonal antibodies were used for assays. The rule of the test – specific anti-IL-5 antibodies which coat the wall of the microplatelet well are bound to IL-5 found in the serum examined. The reaction between the antibodies and IL-5 generates sandwich-type immunological complexes. The number of bound complexes is the measure of serum IL-5 level. The next stage is IL-5 binding to peroxidase. The activity of bound peroxidase is determined photometrically after addition of hydrogen peroxide solution and chromogen. The sensitivity threshold of the method is below 3.0 pg/ml.

Interleukin 6 (IL-6) was determined in 200 µl serum using ELISA method and Quantikine High Sensitivity human IL-6 kit (R&D). Microplatelets, in which wells contained mouse anti-IL-6 monoclonal antibodies, were used for assays. The rule of the test – specific anti-IL-6 antibodies which coat the wall of the microplatelet well are bound to IL-6 found in the serum examined. The reaction between the antibodies and IL-6 yields sandwich-type immunological complexes. The number of bound complexes is the measure of serum IL-6 level. The next stage is IL-6 binding to alkaline phosphatase. The activity of bound alkaline phosphatase is determined after addition of NADPH, which is reduced to NADH. NADH acts as a specific cofactor activating redox-type reactions. In diaphorase-catalyzed reaction NADH reduces tetrazole salt to formazane and NAD^+. NAD^+ is reduced by ethanol in the reaction catalyzed by alcohol dehydrogenase. Colour reaction is proportional to IL-6 reaction. The sensitivity threshold of the method is below 0.094 pg/ml.

Interferone γ (IFN-γ) was determined in 100 µl serum using ELISA method and Quantikine human IFN-γ kit (R&D). Microplatelets with wells containing mouse anti-IFN-γ monoclonal antibodies were used for assays. The rule of the test – specific anti- IFN-γ antibodies which coat the wall of the microplatelet well are bound to IFN-γ found in the serum examined. The reaction between the antibodies and IFN-γ produces sandwich-type immunological complexes. The number of bound complexes is the measure of serum IFN-γ. The next stage is IFN-γ binding to peroxidase. The activity of bound peroxidase is determined photometrically after addition of hydrogen peroxide solution and chromogen. The sensitivity threshold of the method is below 8.0 pg/ml.

Results

The findings revealed that in patients infected with *G. intestinalis* (group G1) the mean IgE level almost twice exceeded the control value, the differences being statistically significant ($p<0.05$). The antiparasitic treatment (groups G2 and G3) only slightly changed the host immunization status (Table 1).

In patients infected with *G. intestinalis* (G1), the mean levels of IL-5 and IL-6 were statistically significantly higher as compared to those noted in healthy subjects ($p<0.001$) (Table 1). After the antiparasitic treatment (G2), the IL-5 concentration was statistically significantly reduced, while IL-6 level increased. However, 2 months after termination of treatment (group G3) both IL-5 and IL-6 concentrations underwent a further decrease compared to G1, but only for IL-5 the difference was statistically significant (Table 1).

In patients infected with *G. intestinalis* (G1), the mean IFN-γ level was almost four times higher than in healthy subjects (C). After the antiparasitic treatment (groups G2 and G3), the mean IFN-γ level was slightly reduced as compared to the values obtained for G1, without statistical significance (Table 1).

Discussion

It has been well documented on animal models that *G. intestinalis* may impair the host immune response. In humans, the infection causes a reduction in IgG and IgA antibodies. Since IgA is involved in the elimination of *G. intestinalis* from the alimentary tract, it seems to play an important role in the immune response [2,13].

Elevated IgE level is a manifestation of increased immunization of the host organism in *G. intestinalis* infection. The IgE level in the infected patients was higher than in healthy subjects and reduced upon application of antiparasitic treatment. Although IFN-γ inhibits IgE production, it was not observed in our patients with *G. intestinalis* infection.

Folia Histochem Cytobiol. 2009:47(1): 94 (93-97)
doi: 10.2478/v10042-009-0013-3

Table 1. The levels of chosen parameters assessing the immune response in patients with *G. intestinalis* infection before treatment (G1), 2 weeks after (G2) and 2 months after (G3) termination of antiparasitic treatment, and in the control group (C). Statistical analysis was performed in examined groups. Values of $p<0.05$ were considered to be significant.

Examined groups	IgE (KIU/l)	IL-5 (pg/ml)	IL-6 (pg/ml)	IFN-γ (pg/ml)
Healthy N=40 C	37.65 ± 27.64	3.59 ± 1.59	2.45 ± 1.44	7.00 ± 3.91
G. intestinalis N=90 G1	65.52 ± 64.66 G1:C, p<0.05*	5.70 ± 2.65 G1:C, p<0.001***	6.33 ± 5.59 G1:C, p<0.001***	25.91 ±13.80 G1:C, p<0.001***
G. intestinalis N=60 G2	61.99 ± 46.89	4.65 ± 2.44 G2:G1, p<0.05*	6.72 ± 3.10 G2:G1, p<0.05*	24.51 ±12.36
G. intestinalis N=43 G3	58.92 ± 39.81	4.16 ± 2.06 G3:G1, p<0.05*	6.25 ± 3.26	23.35 ±11.83

Studies of other authors have shown that in *G. intestinalis* infection the levels of total and specific IgE become elevated [14]. According to Di Prisco *et al.* [14], there exists an allergic type affecting only approximately 2% of patients, with increased synthesis of serous IgE and eosinophilia. IgE induces the cytotoxic functions of platelets through Fc RII/CD23 low affinity receptor for IgE [15]. Souza-Atta *et al.*[16] have observed eosinophilia and a rise in the level of circulating IgE in the course of intestinal parasitic infection (with *e.g. Ascaris lumbricoides*). Cooper *et al.* [17], studying patients infected with *A.lumbricoides* have found *in vitro* a statistically significant increase in the concentrations of IL-4 and IL-5 produced by peripheral blood mononuclear cells (PBMC). This confirms the findings reported earlier by Mahanty *et al.* [18], who observed increased levels of IgE and eosinophilia in patients infected with helminths, and a rise in IL-4 and IL-5 production *in vitro*.

The levels of IL-5 and IL-6 in the course of *giardiosis* were found to be higher than in control group. The treatment applied had no effect on IL-6, but caused a slow normalization of IL-5. Although IL-6 stimulates production of CRP, we observed no increase in the level of this protein in *giardiosis*.

The cytotoxic activity of eosinophils increases under the influence of cytokines released by mast cells, lymphocytes and macrophages (TNF-α , GM-CSF, IL-5). Also macrophages, neutrophils and blood platelets show a cytotoxic action against parasites coated with antibodies [19]. A statistically significant increase in the activity of peroxidase released form eosinophils was induced by IL-2, IFN-γ and GM-CSF. IL-2, like IFN-γ, triggers the release of IL-6 from eosinophils.

Many authors point at the changes in the levels of immunological parameters in parasitic diseases. A considerable increase in IFN-γ and IL-5 concentrations was observed in severe *Schistosoma mansoni* infection [20]. Also *Onchocerca volvulus* infection can cause the immune response from IFN-γ and IL-5. Reaction to microphilaria antigens is particularly sudden in the early stage of the infection [21]. Brattig *et al.* [22] observed a rise in the levels of IL-5 and IL-13 as the response to the administration of the extract soluble *O.volvulus* antigen. These levels were higher in the culture performed in blood samples collected from healthy subjects and significantly lower in patients infected with microfilaria, indicating that the intensity of *O.volvulus* infection affects the levels of IL-5 and IL-13. In patients infected with *Plasmodium falciparum*, possessing LSA 1 (liver stage antigen 1), the production of IL-5, IL-10, IFN-γ and TNF-α is stimulated [23]. Also in the course of *neurocysticercosis* (study performed in the cerebrospinal fluid) the levels of IL-5 and IL-10 are statistically significantly elevated [24].

A report on mice experimentally infected with nematoda *Trichinella spiralis* and *Nippostrongylus brasiliensis* shows that parasitic infections induce the immune and inflammatory response. This response is mediated by cytokines released from Th2 cells (IFN-γ and IL-5) [25]. IFN-γ is produced by activated Th1 cells in the course of allergy development and shows a suppressive action towards synthesis and release of IgE. Through the induction of the production of reactive oxygen and secretion of hydrogen peroxide, IFN-γ stimulates intracellular killing of parasites by macrophages [26]. However, Touil-Boukoff *et al.* [27] have shown that the defense mechanisms in *echinococcosis* first of all involve IFN-γ and IL-6.

Many authors have estimated the effect of IgE, TNF, IFN-γ , IL-6 and C-reactive protein on the cytotoxicity of blood platelets in the course of schistosomosis [6,28-32]. It has been known that the release of toxic substances by platelets depends on the presence of IgE and specific antigens found in a soluble form or on the parasite [34]. Thermal inactivation of IgE causes disappearance of cytotoxic properties of blood platelets, which regain their lost potentials only in the presence of IgE [35]. According to King and Nutman [36] as well as Pritchard [37], parasites (schistosomes and tapeworms) produce and secrete proteases, enzymes that stimulate IgE synthesis and eosinophilia

through the effect on Th2 lymphocytes. On the other hand, by affecting Th1 cells, they regulate and stimulate the production of IgG1, IgG3, activation of the complement system and bacterial phagocytosis. Activation of Th1 and Th2 is strongly connected with the production of specific isotypes of immunoglobulin.

Both our own findings and literature data seem to indicate that parasitic invasions induce immune and inflammatory response, with the involvement of blood platelets and release of IFN-γ , IL-8, TNF-α and IL-5 [25,38].

Conclusions

Increased levels of IgE, IL-5, IL-6 and IFN-γ in *giardiosis* indicate considerable immunization of the host organism. Antiparasitic treatment in *giardiosis* statistically significantly reduces the levels of IL-5 and IL-6.

References

[1] Adam RD. Biology of *Giardia lamblia*. *Clin Microbiol Rev.* 2001;3:447-475.

[2] Faubert G. Immune response to *Giardia duodenalis. Clin Microbiol Rev*. 2000;1:35-54.

[3] Jimenez JC, Fontaine J, Grzych J-M, Dei-Cas E, Capron M. Systemic and mucosal responses to oral administration of excretory and secretory antigens from *Giardia intestinalis. Clin Diagn Labor Immunol*. 2004;11,1:152-160.

[4] Zhou P, Li E, Robertson J, Nash T, Singer SM. Role of interleukin-6 in the control of acute and chronic *Giardia lamblia* infections in mice. *Infect Immunity*. 2003;71,3:1566-1568.

[5] Langford TD, Housley MP, Boes M, Chen J, Kagnoff MF, Gillin FD, Eckmann L. Central importance of immunoglobulin A in host defense against *Giardia spp. Infect Immunity*. 2002;70:11-18.

[6] Pancre V, Monte D, Delanoye A, Capron A, Auriault C. Interleukin-6 is the main mediator of the interaction between monocytes and platelets in the killing of *Schistosoma mansoni. Eur Cytokine Network*. 1990;1:15-19.

[7] Ebert EC. *Giardia* induces proliferation and interferon production by intestinal lymphocytes. *Gut*. 1999;44:342-346.

[8] Weltman JK. Cytokines: regulators of eosinophilic inflammation. *Allergy Asthma Proc*. 2000;21:203-207.

[9] Bliss SK, Marshall AJ, Zhang Y, Denkers EY. Human polymorphonuclear leukocytes produce IL-12, TNF- and the chemokines macrophage-inflammatory protein-1α and -1β in response to *Toxoplasma gondii* antigens. *J Immunol.* 1999;162:7369-7375.

[10] Szeliga J, Goscicka T. Interleukina 12 (IL-12) i jej receptor. *Post Hig Med Dosw*. 2002;56:185-199.

[11] Nutku E, Zhuang Q, Soussi-Gounni A, Aris F, Mazer BD, Hamid Q. Functional expression of IL-12 receptor by human eosinophils: IL-12 promotes eosinophil apoptosis. *J Immunol*. 2001;167:1039-1046.

[12] Majewska AC. *Doświadczalna giardioza: badania porównawcze biologicznych właściwości izolatów Giardia*. Wyd Katedra Biologii i Parazytologii Lekarskiej AM, Poznan, 1996

[13] Hawrelak J. *Giardiasis*: pathophysiology and management. *Altern Med Rev*. 2003;8:129-142.

[14] Di Prisco MC, Hagel I, Lynch NR, Jimenez JC, Rojas R, Gil M, Mata E. Association between giardiasis and allergy. *Ann Allergy Asthma Immunol.* 1998;81:261-265.

[15] Joseph M, Soussi Gounni A, Kusnierz JP, Vorng H, Sarfati M, Kinet JP, Tonnel AB, Capron A, Capron M. Expression and functions of the high-affinity IgE receptor on human platelets and megakaryocyte precursors. *Eur J Immunol.* 1997;27: 2212-2218.

[16] Souza-Atta MLB, Araujo M, D'Oliveira Junior A, Ribeiro-de Jesus A, Almeida RP, Atta AM, Carvalho EM. Detection of specific IgE antibodies in parasite diseases. *Braz J Med Biol Res*. 1999;32:1101-1105.

[17] Cooper PJ, Chico ME, Sandoval C, Espinel I, Guevara A, Kennedy MW, Urban JFJr, Griffin GE, Nutman TB. Human infection with *Ascaris lumbricoides* is associated with a polarized cytokine response. *J Infect Dis.* 2000;182:1207-1213.

[18] Mahanty S, Abrams JS, King CL, Limaye AP, Nutman TB. Parallel regulation of IL-4 and IL-5 in human helminth infections. *J Immunol.* 1992;148:3567-3571.

[19] Polack B, Peyron F, Auriault C. Platelet cytotoxicity against parasites. *Nouv Rev Fr Haematol.* 1991;33:317-322.

[20] de Jesus AR, Silva A, Santana LB, Magalhaes A, de Jesus AA, de Almeida RP, Rego MA, Burattini MN, Pearce EJ, Carvalho EM. Clinical and immunologic evaluation of 31 patients with acute schistosomiasis mansoni. *J Infect Dis.* 2002;185: 98-105.

[21] Cooper PJ, Mancero T, Espinel M, Sandoval C, Lovato R, Guderian RH, Nutman TB. Early human infection with *Onchocerca volvulus* is associated with an enhanced parasite – specific cellular immune response. *J Infect Dis.* 2001;183: 1662-1668.

[22] Brattig NW, Lepping B, Timmann C, Buttner DW, Marfo Y, Hamelmann C, Horstmann RD. *Onchocerca volvulus*-exposed persons fail to produce interferon-gamma in response to *O. volvulus* antigen but mount proliferative responses with interleukin-5 and IL-13 production that decrease with increasing microfilarial density. *J Infect Dis.* 2002;185:1148-1154.

[23] John CC, Sumba PO, Ouma JH, Nahlen BL, King CL, Kazura JW. Cytokine responses to *Plasmodium falciparum* liver-stage antigen 1 vary in rainy and dry seasons in highland Kenya. *Infect Immunity.* 2000;68:5198-5204.

[24] Rodrigues VJr, de-Mello FA, Magalhaes EP, Ribeiro SB, Marquez JO. Interleukin-5 and interleukin-10 are major cytokines in cerebrospinal fluid from patients with active neurocysticercosis. *Braz J Med Biol Res*. 2000;33:1059-1063.

[25] Ishikawa N, Goyal PK, Mahida YR, Li K-F, Wakelin D. Early cytokine responses during intestinalis parasitic infections. *Immunology.* 1998;93:257-263.

[26] Lucey DR, Clerici M, Shearer GM. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. *Clin Microbiol Rev.* 1996;9:532-562.

[27] Touil-Boukoffa C, Sanceau J, Tayebi B, Wietzerbin J. Relationship among circulating interferon, tumor necrosis factor-alpha, and interlekin-6 and serologic reaction against parasitic antigen in human *hydatidosis. J Interferon Cytokine Res*. 1997;17:211-217.

[28] Bout D, Joseph M, Pontet M, Vorng H, Desl D, Capron A. Rat resistance to schistosomiasis: platelet-mediated cytotoxicity induced by C-reactive protein. *Science.* 1986;231: 153-156.

[29] Damonneville M, Wietzerbin J, Pancre V, Joseph M, Delanoye A, Capron A, Auriault C. Recombinant tumor necrosis factors mediate platelet cytotoxicity to *Schistosoma mansoni* larvae. *J Immunol.* 1988;140:3962-3965.

[30] Joseph M, Auriault C, Capron A, Vorng H, Viens P. A new function for platelets: IgE-dependent killing of schistosomes. *Nature*. 1983;303:810-812.

[31] Pancre V, Cesbron JY, Auriault C, Joseph M, Chandenier J, Capron A. IgE – dependent killing of *Brugia malayi* microfi-

Folia Histochem Cytobiol. 2009:47(1): 96 (93-97)
doi: 10.2478/v10042-009-0013-3

lariae by human platelets and its modulation by T cell products. *Int Arch Allergy Appl Immunol*. 1988;85:483-486.
[32] Pancre V, Joseph M, Capron A, Wietzerbin J, Kusnierz JP, Vorng H, Auriault C. Recombinant human interferon-gamma induces increased IgE receptor expression on human platelets. *Eur J Immunol*. 1988;18:829-832.
[33] Pancre V, Joseph M, Capron A, Delanoye A, Vorng H, Auriault C. Characterization of a suppressive factor of platelet cytotoxic functions in human and rat schistosomiasis mansoni. *Clin Exp Immunol.* 1989;76:417-421.
[34] Capron A, Jouault T, Prin L, Joseph M, Ameisen JC, Butterworth AE, Papin JP, Kusnierz JP, Capron A. Functional study of a monoclonal antibody to IgE Fc receptor (Fc epsilon R 2) of eosinophils, platelets, and macrophages. *J Exp Med.* 1986; 164:72-89.
[35] Ameisen JC, Joseph M, Caen JP, Kusnierz JP, Capron M, Boizard B, Wautier JL, Levy-Toledano S, Vorng H, Capron A. A role for glycoprotein IIb-IIIa complex in the binding of IgE to human platelets and platelet IgE-dependent cytotoxic functions. *Br J Haematol.* 1986;64:21-32
[36] King CL, Nutman TB. Biological role of helper T-cell subsets in helminth infections. *Chem Immunol.* 1992;54:136-165.
[37] Pritchard DI. The pro-allergic influences of helminth parasites. *Mem Inst Oswaldo Cruz, Rio de Janeiro* 92. 1997; suppl.2:15-18.
[38] Faccioli LH, Vargaftig BB, Medeiros A, Malheiros A. Cytokines in the modulation of eosinophilia. *Mem Inst Oswaldo Cruz, Rio de Janeiro* 92. 1997;suppl.II:109-114.

Submitted: 21 April, 2007
Accepted after reviews: 9 March, 2008

Folia Histochem Cytobiol. 2009:47(1): 97 (93-97)
doi: 10.2478/v10042-009-0013-3