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EFFECT OF THE COMPOUND L-MIMOSINE IN AN IN VIVO MODEL OF CHRONIC GRANULOMA FORMATION INDUCED BY POTASSIUM PERMANGANATE ($KMNO_4$)

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The plant amino acid L-mimosine has recently been suggested to inhibit cells at a regulatory step in late G_1 phase before establishment of active DNA replication forks. In addition, L-mimosine is an extremely effective inhibitor of DNA replication in chromosomes of mammalian nuclei. In this work, the effect of L-mimosine on chronic inflammation induced by dorsal injections of 0.2 ml of a 1:40 saturated crystal solution of potassium permanganate in mice, was studied. Seven days afterwards, all mice developed a subcutaneous granulomatous tissue indicative of chronic inflammatory response at the site of infection. The intraperitoneal administration of L-mimosine (200 μg/dose) to the potassium permanganate treated mice for 5 consecutive days (the first at the same time of inoculation of the $KMnO_4$), produced a significant decrease in size and weight of the granuloma when compared to mice not treated with L-mimosine (controls). In addition, in all mice treated with L-mimosine, there was a strong inhibition of tumor necrosis factor alpha that was revealed in the serum ($P<0.05$) and in the minced granulomas. Interleukin-6 was not detected in the serum of treated and untreated mice. These findings show for the first time, that L-mimosine may have an anti-inflammatory effect on chronic inflammation and an inhibitory effect on tumor necrosis factor alpha and interleukin-6 generation in supernatant fluids of minced granulomas.

L-mimosine [beta-N (3-hydroxy-4-pyridone)-alpha-amino propionic acid], a major constituent of the tropical legumes *Leucaena glauca Benth* (LBG), is a non protein amino acid, which arrests mammalian cells at a specific point in the late G_1 phase of the cell cycle (1) and suspends DNA transcription in the S phase (2). The cell cycle is also dependent on the effects of retinoic acid (3), and the block by L-mimosine causes an increase in placental alkaline phosphatase (PLAP) activity, which may be related to cell proliferation rate (4). Moreover, L-mimosine completely prevents the uptake of [^{3}H] thymidine into DNA when added to CHO cell line CHOC 400 (5). To explain L-mimosine effects, Trakatelli showed that this compound inhibits the activity of the enzyme serine hydromethyltransferase (L-serine: tetrahydrofolate 5, 10-serine-hydroxymethyl-transferase, SHMT) (2). This enzyme plays a vital role on cell proliferation and immune response, since is very important in the production of one carbon units used in the synthesis of nucleotides, and consequently DNA and RNA. Specifically, from these one carbon units, the C_2 and C_8 of the purine ring (donor=N^{10}, N^{10}-formyltetrahydrofolate), as well as the methyl group of deoxythymidylate (donor=N^5, N^5-

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methylenetetrahydrofolate), are derived. Other investigators demonstrated that arrest of SHMT activity decrease production rate of one carbon units and has a decreased capability to synthesize nucleic acids and proteins (6,7). Addition of mimosine, a potent antagonist of vitamin B_6 coenzymes, in Jurkat cell line (human T-cell leukaemia), which contain high levels of SHMT, inhibits its induction, as well as DNA synthesis, and consequently cell multiplication, a phenomena reversible with the addition of phosphate pyridoxale (PLP) to the culture (2).

Since L-mimosine plays an important role in immunological states, it is pertinent to study its effect in an *in vivo* model of chronic inflammation, where PBMC are abundant and play a pro-inflammatory role (8). In this report, we show the effect of L-mimosine on reducing the size and weight of chronic granuloma tissue induced by potassium permanganate ($KMnO_4$) in the mouse. L-mimosine, which acts as a key element in proliferation and immune response, may be useful as an adjunct in clinical immunosuppressive therapy.

MATERIALS AND METHODS

The experimental animals used in all the experiments were twenty-four male BALB/c mice homogenous in weight and age and raised under the same environmental and feeding conditions. The mice were divided into two groups of twelve mice each and treated as follows. Group 1: receiving saline (100 μl) intraperitoneal (i.p.). Group 2: i.p. administration of 200 μg/100 μl/dose (4) of L-mimosine. Both groups of treated and untreated animals received a dorsal subcutaneous injection (0.2 ml) of a saturated solution of $KMnO_4$ crystals (9,10), which was prepared by adding enough sterile distilled water to dissolve the solid $KMnO_4$ granules and this solution was diluted 1:40 (8,11-13). Twenty-four hours later, and for five consecutive days, the mice were treated i.p. with L-mimosine (200 μg/dose) (treated groups) and saline (control groups). Seven days after the $KMnO_4$ injection, the mice were anesthetized and sacrificed. The granuloma formed at the injection site was carefully measured, calculating the means of the major diameters expressed in millimeters. After the rapid enucleation of the fresh granuloma, the average weight, expressed in grams, was calculated for each group. L-mimosine was purchased from Sigma, Cat no. M.0253, Sigma chimical, Co. P.O. Box 14508 St. Louis, MO 63178 USA.

Tumor Necrosis Factor alpha and interleukin-6 ELISA

The levels of tumor necrosis alpha (TNFα) and interleukin-6 (IL-6) were determined by a specific ELISA purchased from Endogen, Inc., Boston, MA, USA performed on both serum and minced granuloma supernatant fluids from L-mimosine treated and untreated animals 7 days after $KMnO_4$ treatments. The kit was composed of 96 determinations and the assays were performed exactly as recommended by the manufacturer. All samples were assayed in triplicate. Results were expressed as pg/ml.

Granuloma tissue homogenation

Granuloma tissue were surgically excised and placed in ice-cold PBS and homogenized using a Polytron (Brinkmann Instruments, Westbury, N.Y.) for 30 sec. During these procedures, the tissues were kept in ice. The homogenate was centrifuge at 3000 rpm, 4 °C for 10 min and supernatants were analyzed for TNFα and IL-6 production.

Statistical analysis

Data were combined and reported as the means ± SD. The Student's t test for independent means was used to provide a statistical analysis ($P<0.05$ was considered as significant).

RESULTS

Induction of granuloma formation by potassium permanganate and calculation of mean weights of fresh granuloma tissue in L-Mimosine-treated and untreated mice.

In this study, both L-mimosine-treated and untreated mice were assayed for chronic inflammation induced by a dorsal subcutaneous injection of 0.1 ml a saturated solution (1:40 dilution) of $KMnO_4$ crystals. Fig. 1 shows a representative experiment of 24 treated and untreated mice. L-mimosine was given i.p. (200 μg/100 μl bolus injection) for 5 consecutive days, while the untreated mice received only a vehicle. Seven days afterwards, the granuloma was enucleated, weighted and the mean ± S.D. of the weights in 24 mice with or without L-mimosine was estimated. The weights of the granulomas from animals treated with L-mimosine were significantly lower when

compared to the controls (untreated animals), as can be seen in Fig.1.

Fig. 2 shows the inhibitory effect of L-mimosine on fresh mouse granuloma tissue when the mean ± S.D. of its diameters were measured in mm.

Determination of TNFα and IL-6 in serum and supernatants of minced granuloma tissue

In order to assess the anti-inflammatory potential of L-mimosine, we have measured the quantity of the pro-inflammatory proteins TNFα and IL-6 (14-17). Tab. I shows a marked decrease of TNFα levels in the serum of L-mimosine treated mice (control=3352 ± 466 pg/ml vs. 1,351 ± 560 pg/ml to L-mimosine treated). The serum was tested immediately after the animals were sacrificed.

Tab. I. *TNF-alpha production in mouse serum and tissue granuloma.*

TNFα (pg/ml)	Control	L-mimosine	P<
Serum	3352 ± 466 (*)	1351 ± 560	0.01
Supernatant fluids from minced granuloma	1377 ± 218 (*)	436 ± 116	0.05

Effect of L-mimosine μg/bolus given 5 times every 24h, the first at the same time of induction of the granuloma, on TNFα release from serum and granuloma tissue induced by $KMnO_4$ injections (1:40 saturated solution). In order to obtain spontaneous release, the granuloma was minced in cold saline and continuously vortexed for two min. at 4 °C, and tested for TNFα.
The P values (Student's t test) are calculated by comparing L-mimosine-treated animals with L-mimosine-untreated animals. Control (*).

Tab. II. *IL-6 production in mouse serum and tissue granuloma.*

IL-6 (pg/ml)	Control	L-mimosine	P<
Serum	N.D.	N.D.	
Supernatant fluids from minced granuloma	2595 ± 364 (*)	658 ± 148	0.05

Effect of L-mimosine μg/bolus given 5 times every 24h, the first at the same time of induction of the granuloma, on IL-6 release of serum and granuloma tissue induced by $KMnO_4$ injections (1:40 saturated solution). In order to obtain spontaneous release the granuloma was minced in cold saline and continuously vortexed for two min. at 4 °C, and tested for IL-6.
The P values (Student's t test) are calculated by comparing L-mimosine-treated animals with L-mimosine-untreated animals. Control (*).

The inhibitory effect was also observed in the TNFα release in the supernatant of minced fresh granulomas (control=1,377 ± 218 pg/ml vs. 436 ± 116 pg/ml to L-mimosine treated).

IL-6 it was not detected in serum, while in the supernatant fluids from freshly minced granulomas the production of IL-6 was also inhibited by L-mimosine compared to the controls (control=2,595 ± 364 pg/ml vs. 658 ± 148 pg/ml to L-mimosine treated) (Tab. II).

DISCUSSION

Chronic inflammation is characterized by tissue infiltration with a wide variety of inflammatory cells. These cells play important roles in inflammation and natural immunity and function to eliminate microbes and necrosis (11-20). In inflammatory states, when an immune response is responsible for the manifestation of the disease, it becomes desirable to inhibit the immune response. It is now well established that L-mimosine acts as an inhibitor of humoral and cellular immune responses (1,21) since it inhibits the cell cycle progression and DNA replication (22).

Our study demonstrates that i.p. administration of L-mimosine inhibits the weight and the size of the granuloma formation induced experimentally by a saturated solution (1:40) of $KMnO_4$. In addition, in this study we found that TNFα and IL-6 generated by the minced granuloma tissue and in the serum, were strongly inhibited in animals treated with five injections (one every 24h) of L-mimosine 200 μg/dose (the first at the same time of induction of the granuloma). The weight, size and the production of the two mentioned cytokines are related to the degree of inflammation. It is possible that L-mimosine inhibits the granuloma formation and therefore, chronic inflammation through the inhibitory effect on leukocyte function and inflammatory mediators, such as cytokines IL-1 and TNFα, which have important roles in the inflammatory process. It has been previously reported that L-mimosine has an important role in nucleic acid metabolism in immune competent cells and L-mimosine activity affects cellular growth, viral replication, as well as tumor growth, and arrests DNA synthesis at replication forks (23-25).

In a previous study (unpublished results), it was shown that IgG, IgG_1 and IgM, generated in

mice infected with *Trichinella spiralis*, were strongly inhibited in mice treated with L-mimosine (200 μg/dose), suggesting that induction of chronic inflammation could be affected by this amino acid compound. Moreover, since histological analysis of the granuloma tissue documented an accumulation of mononuclear cells (26), which in rheumatoid synovial fluid participate in chronic articular tissue destruction, it is possible that L-mimosine reduces the inflammatory state by inhibiting mononuclear cell activation and proliferation (1).

Recently has been demonstrated that MCP-1, a C-X-C inflammatory chemokine, which acts on macrophage function and regulates protease secretion (27) and MIP-2, a C-C chemokine that directs neutrophils across the epithelial barriers (21), were strongly augmented after infection of *T. spiralis*. The highest serum levels of each of these chemokines were reached at 30 days post-infection (p.i). When infected mice were treated with L-mimosine, it was found that MCP-1 transcription and translation was not completely inhibited by this compound, while MIP-2 transcription and translation was partially inhibited on the 30th and 40th day p.i.

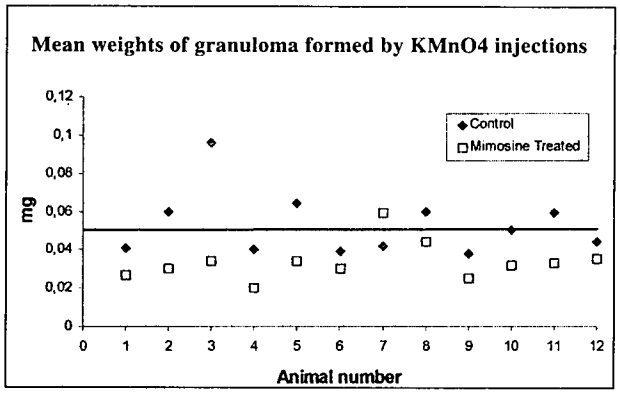

Fig. 1. *These values represent the net weights of granulomas in 24 mice. L-mimosine 200 μg/bolus was given (treated, control) 5 times every 24h, the first, at the same time as the induction of the granuloma. The animals were sacrificed after 7 days from injection of $KMnO_4$. Twelve animals were used as controls (no mimosine) and 12 animals were treated with L-mimosine. P values (Student's t test) were calculated comparing all L-mimosine treated animals with their untreated controls. The P values were < 0.05.*

The protective response to inflammation is mediated by antibodies, neutrophils, macrophages and also by the production of several monokines, such as TNFα and IL-6, produced by different genes that encode non homologous proteins and bind distinct receptors. They appear to play a central role by effecting other cytokines and their serum levels are often correlated to the severity of disease (27).

The generation and secretion of IL-6 involves the early release of TNFα and IL-1 from macrophages, often acting synergistically (28). In fact, they are active on T-cells, B-cells and macrophages and may enhance specific T-cell and antibody mediated responses to the antigen stimuli (29). IL-6 elicits the greatest spectrum of acute phase protein gene activation and protein synthesis *in vitro* and *in vivo*. However, some genes are directly induced by TNFα and IL-6, as others are regulated indirectly via IL-6 (30). The ability of L-mimosine to inhibit TNFα and IL-6 generation in supernatant fluids from minced granuloma induced by $KMnO_4$, may be due to its capacity to

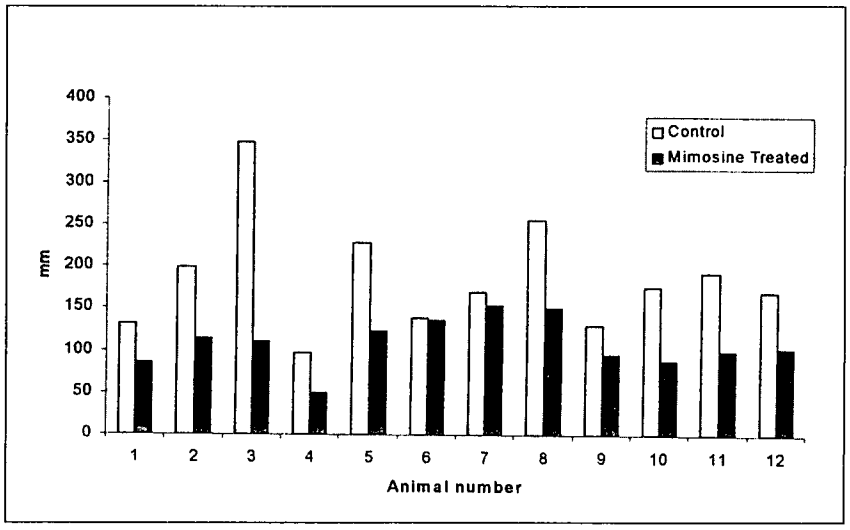

Fig. 2. *The data represent the mean of the measured diameters of granulomas in 24 mice. Mimosine 200 μg/bolus was given (treated, control) 5 times every 24h, the first at the same time as the induction of the granuloma. The animals were sacrificed 7 days after the $KMnO_4$ injection. P values (Student's t test) were calculated comparing all L-mimosine treated animals with their controls. The P values were < 0.05.*

affect mononuclear cells, and to a lesser extent T- and B-cells (28). In fact, these chemokines are polypeptide hormones, which have been demonstrated to be important in immunological reaction and inflammation (31-32). A reduction in activation of leucocytes probably reduces the damaging effects caused by the release of arachidonic acid metabolites, adhesion molecules and inflammatory mediators.

Moreover, it has been recently reported, that L-mimosine acts as an inhibitor of the enzyme SHMT. Since SHMT is very important for cell proliferation and immune responses and plays a vital role for DNA and RNA synthesis leading to T-cell clone expansion (33-34), it is possible that the inhibition of this enzyme by L-mimosine may lead to the arrest of cell proliferation in S phase of the cell cycle, consequently inhibiting cytokine production (35-36). The results in this study demonstrate, that i.p. administration of L-mimosine inhibits the weight and size of experimentally induced granuloma *in vivo* by $KMnO_4$ crystal solution. Moreover, it was found that TNFα and IL-6, which are generated in the granuloma tissue, are dramatically inhibited in animals treated with L-mimosine (200 μg/dose).

Finally, it is worthwhile to mention that L-mimosine decreases nucleic acid synthesis, necessary for the proliferation of cells, by blocking SHMT. Specific inhibitory compounds of SHMT, such as L-mimosine, may prove to have an important role in chronic inflammatory diseases and the present data may suggest that L-mimosine could be beneficial in clinical practice as a chemotherapeutic agent as well as immunosupressor (37-38). However, more studies on L-mimosine should be conducted to fully evaluate biological effects and the sensitivity of this interesting compound.

REFERENCES

1. **T. Krude.** 2000. Initiation of human DNA replication in vitro using nuclei from cells and initiation-competent state. *J. Biol. Chem. 275:13699.*
2. **Trakatelli M.** 1999. The activity of serine-hydromethyl-tranflerase (SHMT) as marker of cell static and immunosuppresor action of different substances (thesis) University of Thessaloniki, p. 1.
3. **Ka-wai-hui E. and B. Yat-ming Yang.** 1993. Cell cycle phase-dependent effect of retinoic acid on the induction of granulocytic differentiation in HL-60 promyelocytic leukemia cells. *FEBS Lett. 318:193.*
4. **Tefler J.F. and C.D Green.** 1993. Placental alkaline phosphate activity in inversely related to cell growth rate in HeLaS3 cervical cancer cells. *FEBS Lett. 329:238.*
5. **Mosca P.J., P.A. Dijwell and J.L. Hamlin.** 1992. The plant amino acid mimosine may inhibit initiation at origins of replication in Chinese hamster cells. *Mol. Cell. Biol. 12:4375.*
6. **Trakatellis A.C. and AE. Axelord.** 1964. Effect of pyridoxine deficiency upon valine incorporation into tissue proteins of the rat. *J. Nutr. 82:483.*
7. **Trakatellis AC. and A.E Axelord.** 1965. Effect of pyridoxine deficiency on nucleic acid metabolism in the rat. *Biochem J. 95:344.*
8. **Conti P., M. Reale, M.R. Panara, S. Frydas, F.C. Placido and R.C. Barbacane.** 1993. Interleukin-1 receptor antagonist inhibits calcium accumulation in in vivo chronic granuloma induced by potassium permanganate. *Calcif. Tissue Int. 52:300.*
9. **Conti P. and M.A. Continenza.** 1980. Decreased ulcerogenic effect of indomethacin in the rat when given in association with diflunisal. *J. Pathol. 131:357.*
10. **Selye H., A. Somogyi and I. Mecs.** 1968. Inhibition of local calcergy by topical application of calciphylactic challengers. *Calci. Tissue Res. 2:67.*
11. .**Smokovitis A.** 1992. Topics of Physiopathology. University Studio Press Thessaloniki.
12. **Frydas S., B. Madhappan and D. Kempuraj.** 2002. Some aspects of parasitology and immunology in general medicine. *Int. J. Imunopathol. Pharmacol. 15:165.*
13. **Huang M., J. Berry, K. Kandere, M. Lytinas, K. Karalis and T.C, Theoharides.** 2002. mast cell deficient W/Wv mice lack stress-induced increase in serum IL-6 levels, as well as in peripheral CRH and vascular permeability, a model of rheumatoid arthritis. *Int. J. Imunopathol. Pharmacol. 15:249.*
14. **Kasahara S., Wago H. and Cooper E. L.** 2002. Dissociation of Innate and Adaptive Immunity by UVB Irradiation. *Int. J. Immunopathol. Pharmacol. 15:1*
15. **S. Frydas, N. Papaioannou, M. Papazachariadou, M. Xatzistilianou, I. Vlemmas, D. Merlitti, M.L. Castellani, C. Schiavone and M. Di Gioacchino.** 2002. A spectrum of antibody (IgG, IgG_1, IgM) response in mice infected with *Trichinella spiralis* treated or not with l-Mimosine. *Int. J. Immunopathol. Pharmacol. 15:19*
16. **M. Di Gioacchino, N. Verna, E. Cavallucci, F. Paolini, R. Caruso, M. Grana, C. Schiavone, A. Di Iorio, S. Ramondo, M. Reale, R. Paganelli and T. C. Theoharides.**

2002. Steroid and antihistamines modulate RANTES release in cultured peripheral blood mononuclear cells of atopic patients. *Int. J. Immunopathol. Pharmacol. 15:27*

17. **T.C. Theoharides, M. Alexandrakis, D. Kempuraj and M. Lytinas.** 2001. Anti-inflammatory actions of flavonoids and structural requirements for new design. *Int. J. Immunopathol. Pharmacol. 14:119.*
18. **A. Conforti, S. Lussignoli, S. Bertani, R. Ortolani, L. Cuzzolin, G. Benoni and P. Bellavite.** 2001. Cytokine and nitric oxide levels in a rat model of immunologic protection from adjuvant-induced arthritis. *Int. J. Immunopathol. Pharmacol. 14:153.*
19. **S. Di Luzio, S. M. Felaco, R.C. Barbacane, S. FrydaS, A. Grilli, M.L. Castellani, M.A. Macrì, M. Di Gioacchino, M.A. De Lutiis, S. Masci, C. Di Giulio, M. Cacchio, and M. Reale.** 2001. Effects of 50 Hz sinusoidal electromagnetic fields on MCP-1 and RANTES generated from activated human macrophages. *Int. J. Immunopathol. Pharmacol. 14:169.*
20. **Kasahara S., Wago H. and Cooper E. L.** 2002. Dissociation of Innate and Adaptive Immunity by UVB Irradiation. *Int. J. Immunopathol. Pharmacol. 15:1*
21. **Hang L., M. Haruoka, W.W. Agace, H. Leffler, M. Burdic, R. Stricter and C. Svanborg.** 1999. Macrophage inflammatory protein-2 is required for neutrophil passage across the epithelial barrier of the infected urinary tract. *J. Immunol. 162:3037.*
22. **Wang Y., J. Zhao, J. Clapper, L.D. Martin, C. Du, E.R. Devore, K. Harkins, D.L. Dobbs and R.M. Bendow.** 1995. Mimosine differentially inhibits DNA replication and cell cycle progression in somatic cells compared to embryonic cells of Xenopus laevis. *Exp. Cell Res. 217:84.*
23. **Gilbert D.M., Neilson A, Miyazawa H, DePamphilis M L, Buzhans W C.** 1995. Mimosine arrests DNA synthesis at replication forks by inhibiting deoxyribonucleotide metabolism. *J. Biol. Chem. 270:9597.*
24. **Polacino P.S., L.M. Pinchuk, S.P. Sidorenko and E.A. Clark.** 1996. Immunodeficiency virus cDNA synthesis in resting T lymphocytes is regulated by T cell activation signals and dendritic cells. *J. Med. Primatol. 25:201.*
25. **Watson P.A., H.H. Hanauske, A. Flint and M. Lalande.** 1991. Mimosine reversibility arrest cell cycle progression at the G1-S phase border. *Cytometry 12:242.*
26. **Conti P., M.R. Panara, S. Frydas, R.C. Barbacane, A. Grill, F.C. Placido, M. Reale and S. Fiore.** 1993. Inhibition of granuloma formation induced by potassium permanganate in the mouse by a specific human recombinant receptor antagonist for interleukin-1 (hrIL-1ra). *Cell Immunol. 147:446.*
27. **Opdenakker G., G. Froyen, P. Fiten, P. Proost and J. Van Damme.** 1993. Human monocyte chemotactic protein-3 (MCP-3): molecular cloning of the DNA and comparison with other chemokines. *Biochem. Biophys Res. Comm. 191:535.*
28. **T.S. Rallis, S. Frydas, N. Soubasis, K.K. Adamama-Moraitou and D. Tontis.** 2002. Monocyte chemotactic protein – 1 in a randomized placebo-controlled study of canine plasmacytic-lymphocytic colitis. *Int. J. Imunopathol. Pharmacol. 15:107.*
29. **Frydas S., E. Karagouni, E. Dotsika, M. Reale, R.C. Barbacane, I. Vlemmas, G. Anogianakis, A. Trakatellis and P. Conti.** 1996. Generation of TNF_, IFN_, IL-6, IL-4 and IL-10 in mouse serum from trichinellosis: effect of the anti-inflammatory compound 4-deoxypyridoxine (4-DPD). *Immunol. Lett. 49:179.*
30. **Toshio Hirano.** Interleukin-6. In: The Cytokine Handbook. A.W. Thomson, Academic Press, Pub. Great Britain 1991; p. 169.
31. **Stadnyk A.W. and J. Gauldie.** 1991. The acute phase protein response during parasitic infection. *Immunol. Today 12:A7-12.*
32. **Dinarello C.A. and J.W. Mier.** 1987. Lymphokines. *N. Engl. J. Med. 317:940.*
33. **Oppenheim J.J. and E K. Durum.** 1988. There is more than one interleukin-1. *Immunol. Today 17:45.*
34. **Axelrod A.E. and A.C. Trakatellis.** 1964. Relationship of pyridoxine to immunological phenomena. *Vitam. Hor. 22:591.*
35. **Axelrod A.E. and A.C. Trakatellis.** 1964. Induction of tolerance to skin homografts by administering splenic cells to pyridoxine deficient mice. *Pro. Soc. Exp. Bio. Med.116:206.*
36. **Conti P.** 1991. Interleukin-1 and interleukin-1 receptor antagonist (IL-1ra). *Ann. Med. Interne 142:521.*
37. **Huang M., J. Berry, K. Kandere, M. Lytinas, K. Karalis and T.C. Theoharides.** 2002. mast cell deficient W/Wv mice lack stress-induced increase in serum IL-6 levels, as well as in peripheral CRH and vascular permeability, a model of rheumatoid arthritis. *Int. J. Immunopathol. Pharmacol. 15:249.*
38. **Chiappini E., M. Peruzzi, L. Galli, G. Calabri and M. de Martino.** 2002. Dose-related IL-6 overproduction by phenobarbital-incubated peripheral blood mononuclear cells in a child with anticonvulsant hypersensitivity syndrome. *Int. J. Immunopathol. Pharmacol. 15:239.*
39. **Biswas S. K. and A. Sodhi.** 2002.Effect of monocyte chemoattractant protein 1 on murine bone marrow cells: proliferation, colony forming ability and signal transduction pathway involved. *Int. J. Immunopathol. Pharmacol. 15:183.*