IMPACT OF IL-33 ON PGD2 GENERATION BY ACTIVATED HUMAN CORD BLOOD-DERIVED MAST CELL: LACK OF EFFECT ON TRYPTASE RELEASE

M. NICOLETTI[1], G. MACCAURO[2], D. TRIPODI[3], A. SAGGINI[4], G. POTALIVO[5], M.L. CASTELLANI[6], F. CONTI[7], M. ROSATI[7], E. TONIATO[6] A. CARAFFA[5], P. ANTINOLFI[5], P. CONTI[6] and T.C. THEOHARIDES[8]

[1]Department of Neurosciences and Imaging, University of Chieti, Italy; [2]Orthopedics Division, Catholic University of Rome, Rome, Italy; [3]Dental School, University of Chieti-Pescara, Chieti, Italy; [4]Dermatology Department, University Tor Vergata, Rome, Italy; [5]Orthopedics Division, University of Perugia, Perugia, Italy; [6]Immunology Division, University of Chieti-Pescara, Chieti, Italy; [7]Gynecology Division, "Santo Spirito" Hospital, Pescara, Italy; [8]Department of Physiology and Pharmacology, Tufts University School of Medicine, New England Medical Center, Boston, MA, USA

Received 28 April, 2012 - Accepted September 13, 2012

Mast cells are important not only in allergic reactions, but also in inflammation and are involved in a variety of responses including the immediate release of potent inflammatory mediators after activation by cross-linking of FcεRI molecules. Prostaglandin D2 (PGD2) is a major cyclooxygenase metabolite of arachidonic acid produced by mast cells and it is released following allergen challenge in allergic diseases. IL-33 is an iflammatory cytokine which is critically involved in the regulation of *in vitro* and *in vivo* cyclooxygenase production, providing a potential therapeutic target for inflammatory disorders. In this study, using human derived umbelical cord blood mast cells, we show that IL-33 (50 ng/ml), and calcium ionophore A 23187 (0.5 μg/ml), compound 48/80 (10^{-5} M) or anti-IgE (10 μg/ml), enhaced the production of PGD2 and this effect was inhibited by indomethacin. However, IL-33 was unable to induce tryptase release in these cells. These effects confirm the inflammatory property of IL-33 by stimulating PGD2 but not tryptase in human mast cells. The inhibitory effect of this new cytokine may have a potential therapeutic response in allergic and inflammatory diseases.

Cytokines are inflammatory and immune-regulatory proteins that play a central role in the pathogenesis of allergic diseases (1-9) and inflammation (10-14). However, some cytokines are anti-inflammatory such as IL-4, IL-10, IL-1 receptor antagonist (IL-1RA) and IL-37. Cytokines of the interleukin-1 (IL-1) family, such as IL-1 alpha/beta, IL-18 and IL-33, have also an important function in host defence, immune regulation, and inflammation (15-20). The mediators released by mast cells, such as histamine, tryptase, serotonin and arachodonic acid products: PGD_2, LTC_4, LTD_4, LTE_4 induce, in general, vasodilation, ronchoconstriction, cellular chemotaxis and increased vascular permeability (21-25). Most of the eicosanoids generated by mast cells are either PGD_2 or LTC_4 (26-29).

Key words: IL-33, mast cells, inflammation, PGD2

Mailing address:
Prof. M. Nicoletti,
Dept. Of Neurosciences and Imaging,
University of Chieti,
Via dei Vestini 31,
Chieti, Italy
e-mail: mauro.nicoletti@uniroma1.it

1721-727X (2012)

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

It has been reported that IL-1beta, IL-18 or IL-33 induced phosphorylation of Erk, p38 and JNK in naïve HDUCBMCs, and IL-33 or IL-1beta, but not IL-18, enhanced the survival of naive HDUCBMCs (30-34). Prostaglandin D2, the major cyclooxygenase metabolite generated from immunologically and non-immunologically stimulated mast cells, is thought to contribute to the pathogenesis of allergic diseases due to its various inflammatory effects (35-38).

The ability of mast cells to generate or release the vasoactive/spasmogenic mediators histamine, PGD2, sulfidopeptide leukotrienes, platelet-activating factor and other factors is thought relevant to immediate broncho-spastic responses in association with mucus secretion and airway edema (39-41). Using human-derived umbilical cord blood mast cell (HDUCBMC), it is pertinent therefore, to evaluate the effect of IL-33 on PGD2 generation in HDUCBMC cultures.

MATERIALS AND METHODS

Cells and materials

Reagents for cell culture were purchased from Sigma-Aldrich. HDUCBMC were cultured in Iscove's Modified Dulbecco's medium supplemented with 10% bovine calf serum, 1.2 mM monothioglycerol, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin either in 25 cm^2 tissue culture plates or in six-well tissue culture plates (Costar). Cells were plated at a density of $0.2x10^6$ per ml taken from the 3-day-old culture grown under the same conditions. Cells were grown in an incubator in a 5% CO_2 and 95% O_2 at 37°C.

Human derived cord blood mast cell isolation, culture and treatment.

Umbilical cord blood was collected in tubes containing 10 U/ml heparin and diluted 1:2 with Dulbecco's phosphate buffered saline (DPBS) from GIBCO BRL (Life Technologies, Grand Island, NY) containing 2 mM ethylenediamine-tetra acetic acid (Sigma). Non-phagocytic mononuclear cells were separated by density-gradient centrifugation using Lymphocyte Separation Medium (LSM). The isolation of hematopoietic stem cells (CD34+) was performed by positive selection of CD34+/AC133+ cells by magnetic associated cell sorting (MACS) using an AC133+ cell isolation kit (Milltenyi Biotec, Auburn, CA). CD133 expression is restricted to a subset of CD34 bright positive stem cells in human cord blood. Mast cells had been obtained by culturing cord blood mononuclear cells in the presence of SCF, IL-6. CD34+ cells were suspended in Iscove's Modified Dulbecco's Medium (IMDM; GIBCO BRL), supplemented with 100 ng/ml rhSCF, 50 ng/ml IL-6, 10% fetal bovine serum, $5x10^{-5}$ M2-Mercaptoethanol, and 1% penicillin-streptomycin (GIBCO BRL) for 12 to 16 weeks. During this culture period, the cells were washed with DPBS every week and resuspended using fresh complete culture medium. Mast cell viability was determined by trypan blue (0.3%) exclusion and observed under light and electron microscope. For sensitization, HDCBMCs were washed with DPBS and plain culture medium (without any growth factors) once in each and resuspended in serum-free complete culture medium, but without IL-6 supplementation. Cells (1 $x10^6$ cells/ml) were then incubated with human myeloma-IgE (2 µg/ml/10^6 cells) at 37°C for 48 h in 24-well Falcon cell culture plates. These sensitized HDUCBMCs were used in all the experiments and treated for 12 h with IL-33, A23187, 48/80 or anti-IgE.

Activation of HCBMC with anti-IgE

Anti-Human IgE (DAKO Rabbit anti-Human IgE, Specific for Epsilon-chains. Code number A 0094; Lot no. 069. Edition 05.07.00) was used in this study at 10 µg/ml. For anti-IgE stimulation experiments, HDUCBMC (10^6 cells/ml) were washed with Iscove's modified Dulbecco's medium and Tyrode's buffer, once in each, and passively sensitized by incubation with human myeloma IgE (2 µg/ml/10^6 cells; Chemicon Inc.) for 48 h in culture medium at 37°C. Cells were then washed two times and resuspended in fresh culture medium. Cells were stimulated with anti-IgE (Dako) at 15 µg/ml in 96-well round bottom culture plates (1 x 10^6 cells/ml in 200 µl medium/ sample) for 12 h at 37°C in 5% CO_2.

Immunoassays for tryptase

Tryptase was measured in the supernatants and cell pellets by UniCAP Tryptase Fluoroenzyme-immunoassay System (Pharmacia Diagnostics AB, Uppsala, Sweden) as reported previously (Schwartz LB 1994). Briefly, anti-tryptase covalently coupled to ImmunoCAP® reacts with the tryptase in the specimen. After washing, enzyme-labeled antibodies against tryptase are added to form a complex. After incubation, unbound enzyme-anti-tryptase is washed away and the bound complex is then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate is measured in the FluoroCount™ 96 microplate reader. The fluorescence is directly proportional to the concentration of tryptase in the sample. To evaluate the test results, the response for the samples is compared directly to the response of the standards. Tryptase was measured in the supernatants and cell pellets by fluoroenzyme-immunoassay (Pharmacia,

Uppsala, Sweden). These peptides were measured in the supernatant and pellet to calculate percent release.

Tryptase release assay: For tryptase measurements, HDUCBMC were sensitized with human myeloma IgE for 48 h in culture medium and were then stimulated for different periods of time in Tyrode's buffer (133 mM NaCl, 4 mM KCl, 0.64 mM KH_2PO_4, 10 mM HEPES, 1 g/L glucose, 1 mM $CaCL_2$, 0.6 mM $MgCl_2$ and 0.03% human serum albumin, pH 7.2). Results were expressed in ng/10^6 cells/ml.

Determination of cell viability

HDUCBMC were harvested, washed in PBS and centrifuged for 5 min at 400x g at room temperature. Cells were then resuspended in culture medium, 0.1% trypan blue solution was added for 5 min at room temperature and the cells were counted using a haemocytometer. Viability was 99%, expressed as percentage of cells which do not take up trypan blue.

Stimulation of HDUCBMC

Calcium ionophore A23187 (0.5 μg/ml), compound 48/80 (10^{-5} M), anti-IgE (10 mg/ml) or IL-33 (50 ng/ml) at different dilutions were made directly in HDUCBMC culture medium. In separate tubes, in each experiment, cells were exposed to the vehicle alone, to determine non-specific release. HDUCBMC (1x10^6 cells/ml) in a six well tissue culture dish were washed with culture medium containing 1 mg/ml bovine serum albumin (BSA) and without Ca++ to reduce spontaneous secretion. They were then sensitized in the same medium for 30 min at 37°C with 2 ml of mouse monoclonal anti-DNP IgE (500 ng/ml). After sensitization the cells were washed again and treated for 60 min at 37°C with 2 ml of DNP-BSA (10 ng/ml) in the same medium, but now supplemented with 0.5 mM calcium to permit secretion. Control samples without IgE were run simultaneously in the presence of 0.5 mM Ca^{++} and these values represented non-specific release. The cells were also activated for 12 h with calcium ionophore A23187 (0.5 μg/ml), compound 48/80 (10^{-5} M), anti-IgE (10 mg/ml) or IL-33 (50 ng/ml) and PGD2 was calculated.

ELISA for PGD2

All the experiments were performed with HDCBMC obtained over about 8 weeks. Briefly, the cells were cultured for 12 h with and without reagents to be tested with anti-IgE (10 mg/ml), 48/80 (10^{-5} M), A23187 (0.5 μg/ml), and IL-33 (50 ng/ml). After incubation, the cells were centrifuged at 4000 rpm. for 8 min. The supernatants were removed and stored at -20° C. The levels of the PGD_2 were determined by a PGD_2-specific ELISA. The kit is composed of 96 determinations and the assays were performed according to the manufacturer's instructions. The ELISA reader was set at 405 nm absorbance and all the samples were read after 1 h after addition of stop solution. The wells were protected from light and incubated at room temperature.

The antiserum anti-PGD_2 (rabbit) was reconstituted in phosphate buffer containing BSA and sodium azide, as recommended by the manufacturer. PGD_2 alkaline phosphatase conjugate was supplied ready to use in bicarbonate buffer containing 0.1% BSA, magnesium chloride and sodium azide at pH 9.8 and stored at 4°C. PGD2 was determined in cell-free supernatants with commercial ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer's directions (sensitivity of the assay, 3 pg/ml).

Statistical analysis

All assays were performed in triplicate. The results were expressed as mean ± SD. Data from three different experiments were combined and reported as the mean ± SD. Student's *t*-test for independent means was used to provide statistical analyses ($p>0.05$ was considered as not significant). Comparisons were made using ANOVA.

RESULTS

Prostaglandin D2 secretion from purified HDUCBMC

The release of prostaglandin D2, from preparations of purified HDCBMC was determined. The production of prostanoid PGD2 was examined after mast cell activation. PGD2 was the prostanoid produced after stimulation of human derived umbilical cord blood mast cells with the IL-33 (50 ng/ml), calcium ionophore A 23187 (0.5 μg/ml), compound 48/80 (10^{-5} M) or anti-IgE (10 μg/ml). The production of PGD2 was apparent after 12 h (Table I) and was still detectble after 48 h (data not shown).

The principal cyclooxygenase products after 12 h was PGD2; while other arachidonic acid compounds appared if any in much lower concentrations (data not shown). Indomethacin (10^{-6} M) markedly inhibited the IL-33 (50 ng/ml), calcium ionophore A 23187 (0.5 μg/ml), compound 48/80 (10^{-5} M) or anti-IgE (10 μg/ml) induced release of PGD2 from HDCBMC and completely suppressed evoked PGD2 synthesis without evident effect on histamine release (data not shown), suggesting that the release of histamine in this model is not dependent on

Table I. *PGD2 production from HDUCBMC (106 cells/ml) following the addition or not of IL-33 (50 ng/ml) and the corresponding samples treated with the calcium ionophore A23187 (0.5 μg/ml), anti-IgE or C48/80*

HDUCBMC PGD2	ng/ml/10^6cells	p
Control	**0.8 +/- 0.5**	*
A23187 (0.5 μg/ml)	**6.5 +/- 1.9**	**0.001**
Anti-IgE (10 mg/ml)	**3.7 +/- 0.4**	**0.001**
C48/80 (10^{-5} M)	**4.7 +/- 1.2**	**0.001**
IL-33 (50 ng/ml)	**2.8 +/- 0.6**	**0.005**
Anti-IgE 10 mg/ml + Indo. 10^{-6} M	**1.7 +/- 1.0**	**N.S.**
A23187 (0.5 μg/ml) + Indo. 10^{-6} M	**0.9 +/- 0.7**	**N.S.**
C48/80 (10^{-5} M) + Indo. 10^{-6} M	**1.2 +/- 0.8**	**N.S.**
IL-33 (50 ng/ml) + Indo. 10^{-6} M	**1.0 +/- 0.8**	**N.S.**

The cells were cultured for 12 h at 37°C, 5% CO2. P values (Student's t-test) are calculated by comparing treated HDUCBMC with untreated cells. The values ± S.D. are representative of three experiments in triplicate.

prostaglandin production. The ability of the cells to secrete PGD2 appears as a cytochemical marker of the *in vitro* mast cells.

Tryptase released from HDUCBMC

HDUCBMCs have tryptase containing granules and receptors for IgE. In this study HDUCBMC were cultured with IL-33 (50 ng/ml) and a classic specific secretagogue anti-IgE, or a non-specific compounds such as calcium ionophore A23187 (0.5 μg/ml) or compound 48/80 (10^{-5} M). Table II shows in three representative experiments the release of tryptase following HDUCBMC treatment and incubated for 60 min with or without IL-33, C48/80, anti-IgE (10 μg/ml) and A23187. Anti-IgE, calcium ionophore A23187 (0.5 μg/ml) or compound 48/80 (10^{-5} M) strongly stimulated tryptase on HDCBMC; while IL-33 was unable to do so.

DISCUSSION

Mast cells play a key role in the induction of allergic disorders, through the release of mediators including histamine, arachidonate products, proteases and several cytokines, which are found in relatively high quantities in these cells (42-46). When activated by diverse stimuli, mast cells mobilize arachidonic acid through cytosolic phospholipase A2, and rapidly generate both prostaglandin D2 (47-51). IL-33 induces biological effects on mast cells and T cells through its receptor IL-33Rβ–chain and may have pro-inflammatory potential effects similar to the cytokines from the same family (52-56). IL-33 is found to be highly expressed in high endothelial venules, tonsils, lymph nodes, and Peyer's patches, in vessels from inflammed tissues, in the intestine of patients with Crohn's disease, and in the synovium

Table II. *Tryptase (ng/ml) release from HCBDMC (106 cells/ml) following the addition or not of IL-33 (50 ng/ml) and the corresponding samples treated with calcium ionophore (A23187) (0.5 μg/ml), C48/80 (10-5 M) or anti-IgE (10 μg/ml).*

(pg/ml)

TREATMENT	10^6 CELLS	p values
Spontaneous	420±100	-
Anti-IgE (10 μg/ml)	610±110	0.005
IL-33 (50 ng/ml)	470.5±90	N.S.
A23187 (0.5 μg/ml)	700±115	0.005
C 48/80 (10^{-5} M)	680±8	0.005

The cells were cultured for 60 min. at 37°C, 5% CO2. P values (Student's t-test) are calculated by comparing untreated HDUCBMC with anti-IgE, calcium ionophore (A23187), C48/80 or IL-33 treated cells. The values ± S.D. are representative of three experiments in triplicate.

of patients with rheumatoid arthritis (57-61). IL-33 is known to enhance experimental allergic inflammation by directly stimulation mast cells to produce inflammatory cytokines (62-65). Therefore, the mast cell is known as a conductor cell in allergic inflammation (66-67). Prostaglandin such as PGD2 is the major prostanoid secreted from the activated mast cell and is an important mediator in the pathogenesis of allergy (68-73). However, the direct effect of IL-33 on PGD2 generation by human mast cells is not known. Here, in this study, we found that IL-33 along with the physiological stimulator anti-IgE or with non-physiological compound A23187 or C48/80, strongly stimulated the production of PGD_2 in HDUCBMC. The stimulatory effect PGD_2 release was completely blocked by a non steroidal anti-inflammatory drug such as indomethacin. We also studied the effect of IL-33 on tryptase release by HDUCBMC and found that IL-33 did not increased the production of tryptase; while anti-IgE, A23187 or C48/80 had a strong stimulator effect.

These results suggest that IL-33 may play an important role in inflammatory diseases by activation PGD2 an arachidonic acid product released from mast cells. These results also indicated that IL-33 may be critically involved in regulation of cyclooxygenase production *in vitro* and probably *in vivo*, providing a potential therapeutic target for inflammatory disorders.

Our study shows that IL-33 activated HDUCBMCs can synthesize significant quantities of PGD2 which may be important in anaphylactic reactions. Prostaglandin D2 produced by mast cells in acute allergic reactions *in vivo* may contribute to the inflammatory reaction, either directly or in synergism with other mediators. The findings from these *in vitro* studies suggest that PGD2 secreted from activated mast cells after IL-33 treatment, may be involved in the formation and/or maintenance of allergic inflammation through its dual receptor systems without involving the activation of tryptase system.

PGD2 is the major cyclooxygenase product released from sensitized human lung and bronchoalveolar lavage mast cells, which possesses a myriad of biological actions relevant to the pathogenesis of asthma (74-79). Thus, modulation of the effects of products of arachidonic acid metabolism by IL-33 may provide a new and more specific treatment for asthma and allergy (80-85).

The localization of the arachidonic acid cascade to the secretory granule explains why the production of eicosanoids is so intimately tied to the process of granule exocytosis.

A significant number of therapeutic approaches for allergies have been designed based on antagonising specific mediators released from mast cells and on selectively inhibiting the activation of these cells (86-89). Here, since IL-33 is a strong stimulator of PGD2 in human mast cells, the inhibition effect of this new cytokine may have a potential therapeutic response in allergic and inflammatory diseases.

REFERENCES

1. Heron I, Berg K, Cantell K. Regulatory effect of interferon on T cells in vitro. J Immunol 1976; 117(4):1370-3.
2. Benucci M, Saviola G, Baiardi P, Manfredi M, Sarzi-Puttini P, Atzeni F. Efficacy and safety of leflunomide or methotrexate plus subcutaneous tumour necrosis

factor-alpha blocking agents in rheumatoid arthritis. Int J Immunopathol Pharmacol 2011; 24(1):269-74.
3. Bellisai F, Morozzi G, Scaccia F, Chellini F, Simpatico A, Pecetti G, Galeazzi M. Evaluation of the effect of Bosentan treatment on proinflammatory cytokine serum levels in patients affected by Systemic Sclerosis. Int J Immunopathol Pharmacol. 2011; 24(1):261-4.
4. Rosato E, Molinaro I, Pisarri S, Salsano F. Subcutaneous immunoglobulin therapy in a patient with myopathic dropped head syndrome and common variable immunodeficiency. J Biol Regul Homeost Agents 2011; 25:667-70.
5. Ruiz-Pérez MV, Sánchez-Jiménez F, Quesada AR, Medina MA. A re-evaluation of the mitogenic effect of serotonin on vascular endothelial cells. J Biol Regul Homeost Agents 2011; 25:13-20.
6. Minai-Fleminger Y, Elishmereni M, Vita F, Soranzo MR, Mankuta D, Zabucchi G, Levi-Schaffer F. Ultrastructural evidence for human mast cell-eosinophil interactions in vitro. Cell Tissue Res 2010; 341(3):405-15.
7. Drago L, Vassena C, Dozio E, Corsi MM, De Vecchi E, Mattina R, Romanò C. Procalcitonin, C-reactive protein, interleukin-6, and soluble intercellular adhesion molecule-1 as markers of postoperative orthopaedic joint prosthesis infections. Int J Immunopathol Pharmacol 2011; 24:433-40.
8. Colombo D, Ammirati E. Cyclosporine in transplantation - a history of converging timelines. J Biol Regul Homeost Agents 2011; 25:493-504.
9. Marchisio P, Bianchini S, Galeone C, et al. Use of complementary and alternative medicine in children with recurrent acute otitis media in Italy. Int J Immunopathol Pharmacol 2011; 24:441-9.
10. Berent-Maoz B, Salemi S, Mankuta D, Simon HU, Levi-Schaffer F. Human mast cells express intracellular TRAIL. Cell Immunol 2010; 262(2):80-3.
11. Nemeth C, Humpeler S, Kallay E, Mesteri I, Svoboda M, Rögelsperger O, Klammer N, Thalhammer T, Ekmekcioglu C. Decreased expression of the melatonin receptor 1 in human colorectal adenocarcinomas. J Biol Regul Homeost Agents 2011; 25:531-42.
12. Mazzoccoli G, Sothern RB, De Cata A, Giuliani F, Fontana A, Copetti M, Pellegrini F, Tarquini R. A timetable of 24-hour patterns for human lymphocyte subpopulations. J Biol Regul Homeost Agents 2011; 25:387-95.
13. Guillén D, Millán O, Brunet M. *In vitro* studies of the immunomodulatory effects of statins alone and in combination with immunosuppressive drugs. Eur J Inflamm 2011; 9:117-24.
14. Sakellaropoulou AV, Hatzistilianou MN, Emporiadou MN, Aivazis VT, Rousso I, Athanasiadou-Piperopoulou F. Evaluation of thyroid gland function in children with obstructive apnea hypopnea syndrome. Int J Immunopathol Pharmacol 2011; 24:377-86.
15. Levi-Schaffer F, Eliashar R. Mast cell stabilizing properties of antihistamines. J Invest Dermatol. 2009; 129(11):2549-51.
16. Di Muzio G, Perricone C, Ballanti E, et al. Complement system and rheumatoid arthritis: relationships with autoantibodies, serological, clinical features, and anti-TNF treatment. Int J Immunopathol Pharmacol 2011; 24:357-66.
17. Cantarini L, Iacoponi F, Lucherini OM, et al. Validation of a diagnostic score for the diagnosis of autoinflammatory diseases in adults. Int J Immunopathol Pharmacol 2011; 24:695-702.
18. Giordano N, Papakostas P, Pecetti G, Nuti R. Cytokine modulation by endothelin-1 and possible therapeutic implications in systemic sclerosis. J Biol Regul Homeost Agents 2011; 25:487-92.
19. Palumbo P, Cinque B, Miconi G, et al. Biological effects of low frequency high intensity ultrasound application on ex vivo human adipose tissue. Int J Immunopathol Pharmacol 2011; 24:411-22.
20. Racciatti D, Gorgoretti V, Sepede G, Gambi F, Pizzigallo E. An Italian study on health-related quality of life and fatigue in patients with chronic fatigue syndrome and patients with chronic HCV virus infection: similarities and differences. Int J Immunopathol Pharmacol 2011; 24:673-81.
21. Bank I, Amariglio N, Reshef A, et al. The hypereosinophilic syndrome associated with CD4+CD3- helper type 2 (Th2) lymphocytes. Leuk Lymphoma 2001;42(1-2):123-33.
22. Nisticò R, Mehdawy B, Piccirilli S, Mercuri N. Paraquat- and rotenone-induced models of

Parkinson's disease. Int J Immunopathol Pharmacol 2011; 24:313-22.
23. Zanna P, Maida I, Turpin Sevilla MC, et al. Molecular characterization of novel melanoma cell lines. J Biol Regul Homeost Agents 2011; 25:239-47.
24. Pierangeli A, Degener AM, Ferreri ML, et al. Interferon-induced gene expression in cervical mucosa during human papillomavirus infection. Int J Immunopathol Pharmacol 2011; 24:217-23.
25. Zoccali C, Chichierchia G, Prencipe U, Nuzzo C, Anelli V, Ferraresi V. A greenstick osteoporotic tibial fracture while on chemotherapy in a 12-year-old girl with osteosarcoma of the knee: does a preventive role exist for agents that reduce iatrogenic bone loss and skeletal-related events? J Biol Regul Homeost Agents 2011; 25:131-5.
26. Berkun Y, Shalit M. Leukotriene antagonists: additional treatment for chronic urticaria? Harefuah 2001; 140(4):347-9.
27. Altorjay I, Veréb Z, Serfozo Z, et al. Anti-TNF-alpha antibody (infliximab) therapy supports the recovery of eNOS and VEGFR2 protein expression in endothelial cells. Int J Immunopathol Pharmacol 2011; 24:323-35.
28. Colombo C, Faelli N, Tirelli AS, et al. Analysis of inflammatory and immune response biomarkers in sputum and exhaled breath condensate by a multi-parametric biochip array in cystic fibrosis. Int J Immunopathol Pharmacol 2011; 24:423-32.
29. Tremolada S, Delbue S, Ferraresso M, Carloni C, Elia F, Larocca S, Bortolani E, Ferrante P. Search for genomic sequences of microbial agents in atherosclerotic plaques. Int J Immunopathol Pharmacol 2011; 24(1):243-6.
30. Murakami M, Taketomi Y, Girard C, Yamamoto K, Lambeau G. Emerging roles of secreted phospholipase A2 enzymes: Lessons from transgenic and knockout mice. Biochimie 2010; 92(6):561-82.
31. Migueres M, Fontaine JF, Haddad T, Grosclaude M, Saint-Martin F, Bem David D, Crestani B. Characteristics of patients with respiratory allergy in France and factors influencing immunotherapy prescription: a prospective observational study (REALIS). Int J Immunopathol Pharmacol 2011; 24:387-400.
32. Li XF, Guo YJ, Wang ML,et al. Inducing-apoptotic activity of the ethanol extract of Duchesnea indica Focke on treatment of herpes simplex encephalitis. Int J Immunopathol Pharmacol 2011; 24:631-8.
33. Guarino F, Cantarella G, Caruso MA, Russo C, Mancuso S, Arcidiacono G, Cacciola RR, Bernardini R, Polosa R. Endothelial activation and injury by cigarette smoke exposure. J Biol Regul Homeost Agents 2011; 25:259-68.
34. Iezzi M, Quaglino E, Cappello P, et al. HCG hastens both the development of mammary carcinoma and the metastatization of HCG/LH and ERBB-2 receptor-positive cells in mice. Int J Immunopathol Pharmacol 2011; 24:621-30.
35. Urade Y, Hayaishi O. Prostaglandin D2 and sleep/wake regulation. Sleep Med Rev 2011; 15(6):411-8.
36. Scaparrotta A, Verrotti A, Consilvio NP, Cingolani A, Di Pillo S, Di Gioacchino M, Verini M, Chiarelli F. Pathogenesis and clinical approaches to anticonvulsant hypersensitivity syndrome: current state of knowledge. Int J Immunopathol Pharmacol 2011; 24:277-84.
37. Tammaro A, Narcisi A, Persechino S, De Marco G, Camplone G. Contact allergy to disperse blue dye in goggles for swimming-bath. Eur J Inflamm 2011; 9:83-84.
38. Shi Q, Gao ZY, Xie F, et al. A novel monoclonal antibody against human CD80 and its immune protection in a mouse lupus-like disease. Int J Immunopathol Pharmacol 2011; 24:583-93.
39. Kubata BK, Duszenko M, Martin KS, Urade Y. Molecular basis for prostaglandin production in hosts and parasites. Trends Parasitol 2007; 23(7):325-31.
40. Sancilio S, Di Giacomo V, Quaglietta AM, et al. TRAIL promotes a pro-survival signal in erythropoietin-deprived human erythroblasts through the activation of an NF-κB/IκBα pathway. J Biol Regul Homeost Agents 2011; 25:375-86.
41. Angelini A, Centurione L, Sancilio SA, Castellani ML, Conti P, Di Ilio C, Porreca E, Cuccurullo F, Di Pietro R. The effect of the plasticizer diethylhexyl phthalate on transport activity and expression of P-glycoprotein in parental and doxo-resistant human sarcoma cell lines. J Biol Regul Homeost Agents 2011; 25:203-211.
42. Tedla N, Lee CW, Borges L, Geczy CL, Arm JP. Differential expression of leukocyte immunoglobulin-

like receptors on cord-blood-derived human mast cell progenitors and mature mast cells. J Leukoc Biol 2008; 83(2):334-43.
43. Luppi F, Covi M, Velluti G, Spagnolo P, Fabbri LM, Richeldi L. Co-trimoxazole effect on human alveolar macrophages of AIDS patients. J Biol Regul Homeost Agents 2011; 25:461-4.
44. Rivetti S, Lauriola M, Voltattorni M, et al. Gene expression profile of human colon cancer cells treated with cross-reacting material 197, a diphtheria toxin non-toxic mutant. Int J Immunopathol Pharmacol 2011; 24:639-49.
45. Miliaras S, Anogeianaki A, Kefala V, Miliaras D, Kokaraki G, Koutsonikolas D, Liangouris J, Anogianakis G. The role of P53 and Bcl-2 proteins in 7, 12- dimethylbenz-(A)-anthracene-induced tumor growth. J Biol Regul Homeost Agents 2011; 25:359-64.
46. Silambarasan G, Ramanathan T, Nabeel MA, Kalaichelvan VK, Kathiresan K, Balasubramanian T. Anti-inflammatory activity of the marine cyanobacterium *trichodesmium erythraeum* against carrageenan-induced paw oedema in wistar albino rats. Eur J Inflamm 2011; 9:53-56.
47. Katz HR, Austen KF. Mast cell deficiency, a game of kit and mouse. Immunity 2011; 35(5):668-70.
48. Sedriep S, Xia X, Marotta F, et al. Beneficial nutraceutical modulation of cerebral erythropoietin expression and oxidative stress: an experimental study. J Biol Regul Homeost Agents 2011; 25:187-94.
49. Failli A, Legitimo A, Mazzoni A, Urbani L, Scatena F, Mosca F, Consolini R. The combination of immunosuppressive drugs with 8-methoxypsoralen and ultraviolet a light modulates the myeloid-derived dendritic cell function. Int J Immunopathol Pharmacol 2011; 24:89-99.
50. Rossi A, Iorio A, Scali E, Fortuna MC, Mari E, Palese E, Greco P, Carlesimo M. Monilethrix treated with minoxidil. Int J Immunopathol Pharmacol.2011; 24(1):239-42.
51. Cassano N, Mastrandrea V, Principi M, Loconsole F, De Tullio N, Di Leo A, Vena GA. Anti-tumor necrosis factor treatment in occult hepatitis B virus infection: a retrospective analysis of 62 patients with psoriatic disease. J Biol Regul Homeost Agents 2011; 25:285-9.
52. Murakami M, Francavilla C, Torselli I, et al. Inactivation of junctional adhesion molecule-A enhances antitumoral immune response by promoting dendritic cell and T lymphocyte infiltration. Cancer Res 2010; 70(5):1759-65.
53. Wu KG, Li TH, Chen CJ, Cheng HI, Wang TY. Correlations of serum Interleukin-16, total IgE, eosinophil cationic protein and total eosinophil counts with disease activity in children with atopic dermatitis. Int J Immunopathol Pharmacol 2011; 24:15-23.
54. Pandolfi F, Cianci R, Casciano F, Pagliari D, De Pasquale T, Landolfi R, Di Sante G, Kurnick JT, Ria F. Skewed T-cell receptor repertoire: more than a marker of malignancy, a tool to dissect the immunopathology of inflammatory diseases. J Biol Regul Homeost Agents 2011; 25:153-61.
55. Mazurek U, Owczarek A, Nowakowska-Zajdel E, Wierzgoń J, Grochowska-Niedworok E, Kokot T, Muc-Wierzgoń M. Statistical analysis of differential gene expression in colorectal cancer using CLEAR-test. J Biol Regul Homeost Agents 2011; 25:279-283.
56. Marotta F, Chui DH, Jain S, Polimeni A, Koike K, Zhou L, Lorenzetti A, Shimizu H, Yang H. Effect of a fermented nutraceutical on thioredoxin level and TNF-α signalling in cirrhotic patients. J Biol Regul Homeost Agents 2011; 25:37-45.
57. Penrose JF, Baldasaro MH. Leukotriene C4 synthase: a candidate gene for the aspirin-intolerant asthmatic phenotype. Allergy Asthma Proc 1999; 20(6):353-60.
58. Tarantino G, Colao A, Capone D, et al. Circulating levels of cytochrome c, gamma-glutamyl transferase, triglycerides and unconjugated bilirubin in overweight/obese patients with non-alcoholic fatty liver disease. J Biol Regul Homeost Agents 2011; 25:47-56.
59. D'Alimonte I, Nargi E, Mastrangelo F, et al. Vascular endothelial growth factor enhances *in vitro* proliferation and osteogenic differentiation of human dental pulp stem cells. J Biol Regul Homeost Agents 2011; 25:57-69.
60. Failli A, Consolini R, Legitimo A, Orsini G, Romanini A, Spisni R, Castagna M, Miccoli P. Evaluation of *in*

vitro cytotoxicity of oxaliplatin and 5-fluorouracil in human colon cancer cell lines: combination versus sequential exposure. J Biol Regul Homeost Agents 2011; 25:575-88.

61. Braido F, Schenone G, Pallestrini E, Reggiardo G, Cangemi G, Canonica GW, Melioli G. The relationship between mucosal immunoresponse and clinical outcome in patients with recurrent upper respiratory tract infections treated with a mechanical bacterial lysate. J Biol Regul Homeost Agents 2011; 25:477-85.
62. Penrose JF, Austen KF. The biochemical, molecular, and genomic aspects of leukotriene C4 synthase. Proc Assoc Am Physicians 1999; 111(6):537-46.
63. Dell'Edera D, Guanciali Franchi P, Lioi MB, et al. Biochemical and molecular characterization of von Willebrand disease type 2N in a pregnant patient who gave birth under analgesia with remifentanil. J Biol Regul Homeost Agents 2011; 25:469-75.
64. Negrev N, Tashev R, Radev R, Anogeianaki A, Ivanova M. Hormones of hypothalamic-pituitary-thyroid axis are significant regulators of synthesis and secretion of vitamin K-dependent plasma coagulation factors. J Biol Regul Homeost Agents 2011; 25:21-26.
65. Artini M, Scoarughi GL, Papa R, et al. A new anti-infective strategy to reduce adhesion-mediated virulence in Staphylococcus aureus affecting surface proteins. Int J Immunopathol Pharmacol 2011; 24:661-72.
66. Marzano AV, Cugno M, Trevisan V, Lazzari R, Fanoni D, Berti E, Crosti C. Inflammatory cells, cytokines and matrix metalloproteinases in amicrobial pustulosis of the folds and other neutrophilic dermatoses. Int J Immunopathol Pharmacol 2011; 24:451-60.
67. Verratti V, Brunetti L, Tenaglia R, et al. Physiological analysis of 8-Iso-PGF2 alpha: a homeostatic agent in superficial bladder cancer. J Biol Regul Homeost Agents 2011; 25:71-76.
68. Barrett NA, Austen KF. Innate cells and T helper 2 cell immunity in airway inflammation. Immunity 2009; 31(3):425-37.
69. Pizzicannella J, Rabozzi R, Trubiani O, Di Giammarco G. Histidine-tryptophan-ketoglutarate solution helps to preserve endothelial integrity of saphenous vein: an immunohistochemical and ultrastructural analysis. J Biol Regul Homeost Agents 2011; 25:93-99.
70. Antonelli A, Fallahi P, Ferrari SM, Di Domenicantonio A, Moreno M, Lanni A, Goglia F. 3,5-diiodo-Lthyronine increases resting metabolic rate and reduces body weight without undesirable side effects. J Biol Regul Homeost Agents 2011; 25:655-60.
71. De Amici M, Villani MA, Milanesi E, Rossini B, Barco S, Gerletti M, Ciprandi G. Adverse reactions to anaesthetics prevented by the use of specific laboratory tests Eur J Inflamm 2011; 9:79-81.
72. Re M, Magliulo G, Tarchini P, Mallardi V, Rubini C, Santarelli A, Lo Muzio L. p53 and BCL-2 over-expression inversely correlates with histological differentiation in occupational ethmoidal intestinal-type sinonasal adenocarcinoma. Int J Immunopathol Pharmacol 2011; 24:603-9.
73. Carlesimo M, Narcisi A, Cortesi G, Mari E, Fidanza L, De Marco G, Rossi A, Camplone G. An 18-year follow-up of a case of D-penicillamine-induced Elastosis perforans serpiginosa. Int J Immunopathol Pharmacol.2011; 24(1):257-9.
74. Austen KF, Maekawa A, Kanaoka Y, Boyce JA. The leukotriene E4 puzzle: finding the missing pieces and revealing the pathobiologic implications. J Allergy Clin Immunol. 2009; 124(3):406-14.
75. Wu KG, Li TH, Chen CJ, Cheng HI, Wang TY. A pilot study evaluating the clinical and immunomodulatory effects of an orally administered extract of Dendrobium huoshanense in children with moderate to severe recalcitrant atopic dermatitis. Int J Immunopathol Pharmacol 2011; 24:367-75.
76. Sedriep S, Zhong K, Nakanishi K, Sweed H, Chui DH, Yang H, Xia X, Catanzaro R, Zhou L, Marotta F. Advantage of carbonate- versus citrate-based alkalinization on bone metabolism in moderately exercising aged male rats fed an acidogenic diet. J Biol Regul Homeost Agents 2011; 25:341-9.
77. Orciani M, Morabito C, Emanuelli M, Guarnieri S, Sartini D, Giannubilo SR, Di Primio R, Tranquilli AL, Mariggiò MA. Neurogenic potential of mesenchymal-like stem cells from human amniotic fluid: the influence of extracellular growth factors. J Biol Regul Homeost Agents 2011; 25:115-30.
78. Patella V, Incorvaia C, Ricciardi L, Florio G, Saija A, Frati F, Gangemi S. The adhesion molecule ICAM-

1 is overexpressed in patients with hymenoptera venom allergy and decreases after ultrarush venom immunotherapy. J Biol Regul Homeost Agents 2011; 25:465-8.

79. Gallenga PE, Del Boccio M, Rapinese M, Di Iorio A, Toniato E, Martinotti S. Molecular approach by PCR is the best method to detect the presence of Chlamydia trachomatis and to define the true agent of ocular bacterial inflammation. Int J Immunopathol Pharmacol 2011; 24:285-96.
80. Arm JP. Leukotriene generation and clinical implications. Allergy Asthma Proc. 2004; 25(1):37-42.
81. Losi M, Bocchino M, Matarese A, et al. Role of the quantiferon-TB test in ruling out pleural tuberculosis: a multi-centre study. Int J Immunopathol Pharmacol 2011; 24:159-65.
82. Riccioni G, Scotti L, Di Ilio E, et al. Lycopene and preclinical carotid atherosclerosis. J Biol Regul Homeost Agents 2011; 25:435-41.
83. Battelli MG, Ravaioli M, Musiani S, Scicchitano V, Grazi GL, Bolognesi A. Increased serum level of xanthine oxidoreductase in liver transplanted patients. J Biol Regul Homeost Agents 2011; 25:77-84.
84. Sharma JN, Kesavarao U. The effects of captopril on cardiac regression, blood pressure and bradykinin components in diabetic Wistar Kyoto rats. Int J Immunopathol Pharmacol 2011; 24:337-43.
85. Veda P. Why are neutrophils polymorphonuclear? Eur J Inflamm 2011; 9:85-93.
86. Almerigogna F, Fassio F, Giudizi MG, et al. Natural killer cell deficiencies in a consecutive series of children with herpetic encephalitis. Int J Immunopathol Pharmacol 2011; 24:231-8.
87. Malerba M, Ragnoli B, Salameh M, Sennino G, Sorlini ML, Radaeli A, Clini E. Sub-clinical left ventricular diastolic dysfunction in early stage of chronic obstructive pulmonary disease. J Biol Regul Homeost Agents 2011; 25:443-51.
88. Nenna R, Papoff P, Moretti C, et al. Detection of respiratory viruses in the 2009 winter season in Rome: 2009 influenza A (H1N1) complications in children and concomitant type 1 diabetes onset. Int J Immunopathol Pharmacol 2011; 24:651-9.
89. Germano V, Picchianti Diamanti A, Ferlito C, Podestà E, Salemi S, Migliore A, D'Amelio R, Laganà B. Cyclosporine A in the long-term management of systemic lupus erythematosus. J Biol Regul Homeost Agents 2011; 25:397-403.