Lymphology 21 (1988) 144-151

Modulation of Mouse Spleen Natural Killer (NK) Cell Activity by Beta-Interferon, Interleukin-1, and Prostaglandins

C.L. Voiculescu, L. Rosu, S. Rogoz

Department of Microbiology, Faculty of Medicine, Craiova, Rumania

ABSTRACT

By using CBA/J mice as a source of effector cells and Yac-1 lymphoma line as "target" cells, the natural killer (NK) cell activity was assayed following both in vivo *and* in vitro *immunomodulation [beta-interferon (IF), interleukin-1 (IL-1), indomethacin (IND), prostaglandin-E_2 (PGE)].*

Only IF/IND and IL-1/IND mixed in vivo *led to a significant augmentation of NK cell activity. If exposed* in vitro *to IF or to IL-1, control group-derived spleen NK cells exhibited increased cytotoxic activity whereas PGE-exposure only was followed by a lower cytotoxic level as expressed both in % cytotoxicity curves and in lytic units 20%/10^7 effector cells.*

On the other hand, PGE seemed to activate the "nonspecific suppressor" (NSS) cell subset in its inhibitory effect about NK cells as tested in vitro *in several NK/NSS cell mixtures at different ratios. IF, but not IL-1 diminished the NSS-cell-induced suppressive activity. Pre-exposure of NK/NSS cell mixtures to IF followed by PGE exposure, did not prevent PGE-dependent NSS cell activation.*

Interferons, especially beta or alpha moeities, stimulate natural killer (NK) cell activity (1-7). Although not as firmly established, some data suggest that interleukin-1 monokine as well as interleukin-2 lymphokine are also able to stimulate NK cell cytotoxicity (8-13). On the other hand, *in vitro* experiments carried out with several E prostaglandin preparations as well as *in vivo* in conjunction with some prostaglandin inhibitory drugs, exhibit a prostaglandin-dependent diminution of NK cell activity (14). However, there is little information about synergistic or antagonistic effects exerted by different immunomodulating agents (i.e., macrophage or lymphocyte-derived prostaglandins) on natural cytotoxicity as tested in humans or in experimental animals (1,4,7,12,15).

In this context we examined the influence of several *in vivo* immunomodulating treatments (beta-interferon, interleukin-1, and indomethacin respectively) on mouse spleen NK cell activity; the behavior of several cytotoxic levels after *in vitro* exposure of mouse spleen NK cells to beta-interferon, interleukin-1, or E_2-prostaglandin, and the exchanges in nonspecific suppressor (NSS)/NK cell balance related to several *in vitro* stimulating or inhibiting immunomodulation procedures.

MATERIALS AND METHODS

Animals

CBA/J "inbred" mice (Cantacuzino Institute, Bucharest, Rumania), 40 or 10

days old were used respectively.

Immunomodulators

Beta-interferon (IF). Murine embryo cell line *Swiss-12* (16) was infected with vaccinia virus (standard preparation L_{546}/78, Cantacuzino Institute, Bucharest, Rumania), at a rate of 20 CPD_{50}/cm^2 of culture area. Cultures were grown in Falcon Petri dishes, in the presence of Eagle's MEM medium supplemented with 10% calf serum (Gibco). Following virus inoculation, each culture was incubated for 96 hrs at 37°C in the same medium without serum. All supernates were collected, dialyzed against glycocol/HCl buffer (0.1M, pH 2.2) for 24 hrs at 4°C, redialyzed twice against phosphate buffer saline-PBS (0.07M, pH 7.2) each for 24 hrs at 4°C. The samples were subsequently centrifuged at 250g for 10 min (Janetzki K_{70}), pellets discarded, and supernates harvested, collected, and considered as non-purified interferon preparations. In order to obtain semi-purified specimens, affinity chromatography by means of Concanavalin A/Sepharose B columns (Pharmacia, Uppsala, Sweden) was effectuated (according to Wietzerbin et al., 1979 (17)). Titration of IF antiviral activity was made in the same cell substratum in the presence of logarithmic dilutions of vaccinia virus (IF titer expressed in inhibitory units/mg of protein). In further experiments only IF preparations exhibiting at least 5 x 10^7 inhibitory units (IU)/mg of protein were used.

Interleukin-1 (IL-1). Non-purified IL-1 specimens were obtained in mouse spleen macrophage cultures, prepared from spleen total cells suspended in Eagle's MEM supplemented with 10% calf serum (Gibco) and plated in Falcon dishes at 37°C for 30 min (18). Adherent cells were thereafter cultured in the same medium but without serum for 48 hrs at 37°C in the presence of *E. coli* $O_{111}B_4$-derived lipopolysaccharide (LPS) (Sigma Chemical Co., St. Louis, Missouri, USA), 0.07mg/cm^2 of culture area (19). When the incubation time was over, supernates were collected and concentrated 10x by means of polyvinylpyrrolidone (Sigma Chemical Co., St. Louis, Missouri, USA), molecular weight 40,000. Purified IL-1 was obtained by Sephadex G_{50} chromatography (165 x 5.6cm column at 4°C in 0.9% NaCl, pH 5.6 to 5.9; collection of the 15,000 dalton molecular weight peak; dialysis against distilled water) (8). Purified IL-1 specimens were titrated as regard their stimulating activities by means of thymocyte proliferation assay (19). All titres were expressed in units 50% (U_{50}): $1U_{50}$ = reciprocal of IL-1 dilution that produced half of the maximum response to phytohemagglutinin-M in thymocyte cultures, stimulated and treated with ^3H-thymidine. In further experiments, an IL-1 preparation with a specific activity of 1 x 10^7 U_{50}/mg of protein was used.

Prostaglandin-E (PGE). A standard preparation of prostaglandin-E_2-dried powder from *Plexaura homomalla* coral (Sigma Chemical Co., St. Louis, Missouri, USA) was tested (work solutions: 5,000 and 1,000 nM/ml of PBS, respectively).

Prostaglandin-inhibitor. A commercial preparation of indomethacin (IND) pulvis (Sigma Chemical Co., St. Louis, Missouri, USA) was used. The substance was dissolved in PBS so as to obtain a work solution of 250 and 125μg/ml, respectively.

NK cell assays

Obtaining of mouse spleen NK cells. Total spleen cells, obtained by teasing, resuspended in Eagle's MEM supplemented with 10% calf serum (Gibco), filtered by gauze and centrifuged by density gradient with Percoll (Pharmacia, Uppsala, Sweden), at different concentrations in Eagle's MEM + 10% calf serum--starting with 56.6% (v/v) Percoll and grading by 4.5% concentration diminutions to 38.6% Percoll at the top--were layered into a 20ml round-bottomed glass tube (1 x 10^6 cells/1ml layered on the top; centrifugation at 300g for 45 min at 20°C; collection of each cell fraction

Table 1
Cytotoxic Activity of Several Mouse Spleen Cell Fractions

Cell fraction	% of cells recovered	NK cell cytotoxic activity (lytic units 20%/10^7 recovered effector cells)
Total spleen cells input	100.0	16
F - 0	7.6	68
F - 1	15.4	228
F - 2	26.8	75
F - 3	37.0	2
F - 4	12.5	<0.80
F - 5	0.7	-

and washing twice with fresh medium) (20). All fractions (from F-0 to F-6) were tested as concerns their cytotoxic activities in 4-hr cytotoxic assay (see *Cytotoxic activity* below). The maximum of activity was obtained in F-1 fraction *(Table 1)*. Subsequently, the following controls for mouse NK cells into the F-1 cell fractions were performed (21): amount of Ig^+ immunoglobulin-bearing) cells; % cytotoxicity, following incubation for 30 min in the presence of D-mannose (Serva, Praha), 1% solution in PBS, or in the presence of trypsin (Difco Labs, Detroit, Michigan, USA), 0.125% in Tris buffer 0.025 M pH 7.6 at 37°C respectively, at a rate of 100:1 effector/"target" cell ratio. F-1 spleen cells were Ig^+ only in 0-5% (confidence interval 95% for n=250) and sensitive to D-mannose and trypsin *in vitro* treatment (% cytotoxicity 21.50 ± 12.40 and 37.00 ± 5.29 respectively, as compared to F-1 control cells: 81.50 ± 2.64) ("positive" markers for murine spleen NK cells).

"Target" cells. Yac-1 cell line (Maloney virus-induced lymphoma of A/Sn origin) was used (22).

Cytotoxicity assay. "Target" cells (1 x 10^7 cells/0.2ml) derived from cultures in exponential growth phase were incubated with 200μCi of $Na^{51}CrO_4$ (Amersham Corp., Arlington Heights, Illinois, USA) for 1 hr at 37°C. The labeled cells were thoroughly washed and adjusted to a final amount of 1 x 10^5 cells/ml of Eagle's MEM. Portions of different specimens of effector cells (0.1ml) were added to 1 x 10^4/0.1ml radiolabeled Yac-1 cells in round-bottomed microtiter plates (Lindbro Chemical Co., Hamden, Connecticut, USA), at different effector/"target" (E/T) ratios, i.e., 100:1, 50:1, 25:1, and 12.5:1 respectively. All plates were centrifuged at 100 g for 3 min and incubated for 4 hrs at 37°C in a humified incubator with 5% CO_2. Culture supernates were harvested and radiometrically measured in a Packard Tri-Carb gamma counter. The data were presented in three ways as follows:

1) *% cytotoxicity* (mean ± S.D.), scored by the formula:

$$\frac{\textit{experimental cpm - spontaneous cpm}}{\textit{maximal cpm - spontaneous cpm}} \times 100$$

according to Riccardi et al, 1983 (22).

2) *"Positive" cultures* (mean for 96-wells scored): as "positive" were considered only cultures exceeding the mean background value ("target" cells only) by at least 3 standard deviations (S.D.) (according to Wei-Feng, 1982 (23)).

3) *Lytic units 20%/10^7* effector cells scored from linear parts of the cytotoxic curves at different E/T ratios (24).

4) "In vivo" *immunomodulating schedules.* Six 40-day-old mouse groups (10 mice per group) were used, *"in vivo"* treated with several schedules as follows: *C-group* (control): PBS, 0.2ml/mouse/in-

Table 2
Cytotoxicity Levels in Spleen NK Cells Arising from Several Mouse Groups *In Vivo*

E/T Ratio	Cytotoxicity % (mean ± S.D., n=4)					
	C	IF	IL-1	IND	IF/ IND*	IL-1/ IND**
100:1	61.75 ± 2.36	60.50 ± 4.20	59.50 ± 4.20	60.50 ± 5.80	77.50 ± 3.69	75.75 ± 4.35
50:1	51.25 ± 2.29	50.00 ± 6.05	50.75 ± 6.60	50.25 ± 7.93	74.50 ± 10.00	53.50 ± 5.51
25:1	45.00 ± 4.76	42.25 ± 5.56	39.75 ± 8.80	42.50 ± 10.85	61.25 ± 2.99	44.00 ± 5.89
12.5:1	35.25 ± 4.11	34.50 ± 5.97	34.00 ± 9.20	33.75 ± 7.50	41.25 ± 5.68	34.50 ± 5.26

*Spontaneous release ("target" cells only): 2.25 ± 0.96
**Significant differences in the test "t" (Student):
-between IF/IND and C groups: $p<0.02$ at 100:1 E/T ratio; $p<0.05$ at 50:1 E/T ratio; $p<0.05$ at 25:1 E/T ratio
-between IF/IL-1 and C groups: $p<0.05$ at 100:1 E/T ratio

jection by intraperitoneal route twice a week for 3 weeks (6 injections); *IF-group*: Beta-interferon, 1 x 10^5 I.U./0.2ml/mouse/injection, under the same conditions as in C-group; *IL-1-group*: interleukin-1, 2 x 10^2 U_{50}/0.2ml/injection, under the same conditions as in C-group; *IND-group*: Indomethacin, 25μg/0.2ml/mouse/injection under the same conditions as in C-group; *IF/IND-group*: a mixture of beta-interferon and indomethacin (final concentration: 1 x 10^5 I.U. and 25μg, respectively/0.2ml/mouse/injection, under the same conditions as in C-group; *IL-1/IND-group*: a mixture of interleukin-1 and indomethacin (final concentration: 2 x 10^2 U_{50} and 25μg , respectively/0.2ml/mouse/injection, under the same conditions as in C-group. Three weeks after beginning of experiments, the cytotoxic activities of several NK cell specimens were tested, by means of the 4-hrs cytotoxicity assay (see above, "cytotoxicity assay").

5) In vitro *immunomodulating schedules*. *C-group* derived NK cells (1 x 10^7/ml) were divided into four portions. Each portion was centrifuged for 5 min at 150 g, pellets resuspended to the initial volume in several solutions as follows: Eagle's MEM *(control)*; *IF* (10 I.U./effector cell); *IL-1* (2 U_{50}/effector cell); *PGE* (1 x 10^{-4} nM/effector cell). Each sample was incubated for 3 hrs at 37°C, then washed, resuspended in fresh medium, and adjusted to the optimal amounts as to obtain several E/T ratios in the 4-hrs cytotoxicity assay.

6) *NK/NSS relationships as tested following several immunomodulating procedures* in vitro. *a. Obtaining of NSS cells.* Nylon-adherent spleen cells belonging to 10-day-old CBA/J mice, adjusted finally to 1 x 10^9 cells/ml of Eagle's MEM as NSS cells were considered, according to other data (1,25). *b. Experiment.* Several NSS/NK cell mixtures from 20:1 to 1:1 ratios, as well as NK or NSS cells alone at the same amount were made, each of them being then incubated in the presence of different immunomodulators as follows: *medium alone (control)*; *IF* (0.5 I.U./NSS cell); *IL-1 (0.1 U_{50}*/NSS cell); *PGE* (2.5 x 10^{-5} nM/NSS cell). All

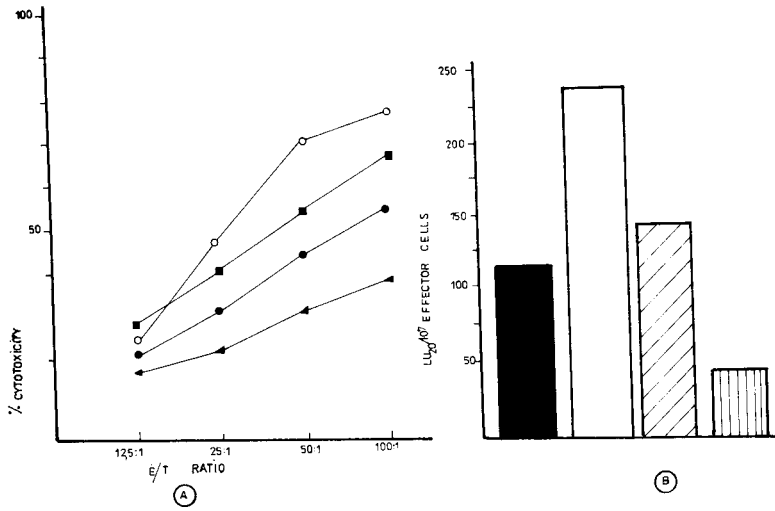

Fig. 1. NK cell activity following in vitro *exposure to several immunomodulators. (A) % cytotoxicity (mean of "positive" cultures from 96 wells containing similar samples). Solid circles--control; open circles--IF; solid squares--IL-1; solid triangles--PGE. (B) Lytic units 20%/10^7 effector cells LU_{20}. Solid bar--control; open bar--IF; angled hatched bar--IL-1; vertical hatched bar--PGE.*

samples were incubated for 3 hrs at 37°C before tested as to their cytotoxicity in the presence of "target" cells. In addition, a "*pre-IF*" sample was made, consisting of NSS/NK cell mixtures pre-incubated with IF (0.5 I.U./NSS cell) for 1 hr, washed twice and incubated with PGE (2.5×10^{-5} nM/NSS cell) for 3 hrs at 37°C. All results were expressed in *% cytotoxicity* (mean ± S.D.) for 10 similar samples, respectively. Mean of all S.D. values ± S.D. exhibited a limited range of 5.82 ± 2.21 whereas background values did not exceed 1% (0.98 ± 0.59).

RESULTS

NK cell cytotoxic levels following in vitro *administration of several immunomodulating agents*

As seen in *Table 2*, IF-, IL-1, and IND treatment alone did not alter the cytotoxic curves at any E/T ratio as compared to the controls. Mixed IF/IND administration *in vivo* led to an increase of NK cell activity at 100:1, 50:1, and 25:1 E/T ratios ($p<0.02$, $p<0.05$, and $p<0.05$ respectively). Mixed IL-1/IND treatment of mice exhibited an augmentation of the natural cytotoxicity only at the highest E/T ratio ($p<0.05$).

NK cell cytotoxic patterns following in vitro *incubation of the effector cells with several immunomodulators*

When expressed as % cytotoxicity *(Fig. 1A)*, the data suggest that at least at 100:1 and 50:1 E/T ratios IF or IL-1 *in vitro* exposure of the effector cells were followed by increased target" cell lysis. At the same E/T ratio, PGE depressed the rate of isotope release. Presentation of synthetic data related to this kind of experiment *in vitro* by scoring the *lytic units 20%/10^7 effector cells (Fig. 1B)* show that IL-1 and especially IF-pre-exposure of NK cells *in vitro* augmented their lytic abilities (increase with about 50% of LU_{20} values in IL-1 sample and with more than 200% in IF sample, respectively). On the other hand, PGE depressed strongly the NK cell activity (LU_{20} values with 27% less as compared to the control).

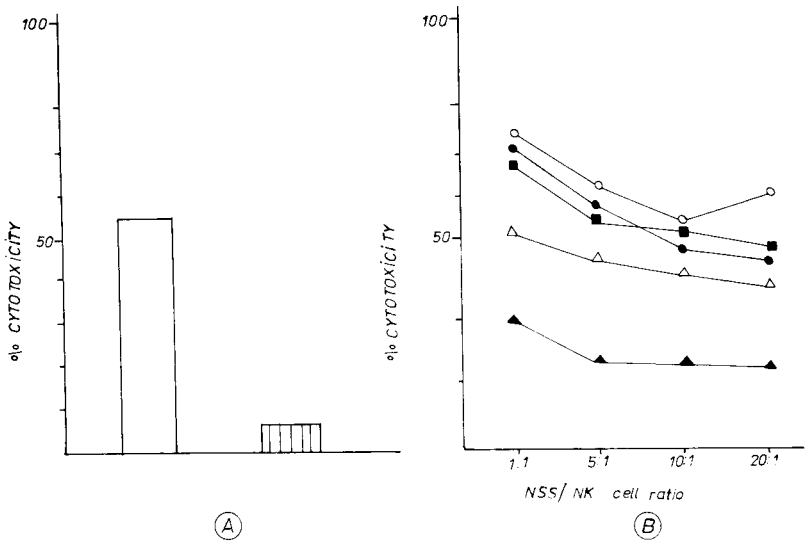

Fig. 2. Suppressive effects exerted by NSS cells on NK cell cytotoxic levels related to several in vitro *exposures to different immunomodulating agents. (A) Controls: open bar--NK cells; hatched bar--NSS cells. (B) NSS/NK cell mixtures at different ratios: solid circles--control--NSS/NK; open circles--IF;solid squares--IL-1; solid triangles--PGE; open triangles--pre-IF/PGE.*

Influence of beta-interferon, interleukin-1, and prostaglandins on the inhibitory relation NSS/NK cells in vitro (Fig. 2)

A gradual decrease of the cytotoxic level in control NSS/NK cell mixtures occurred, related to augmentation of the NSS/effector cell ratio. Only NSS cells failed to exhibit significant cytotoxicity in the presence of labeled "target" cells. IL-1 seems not to influence NSS/NK balance behavior under our experimental conditions. In exchange, a noteworthy preventing effect exerted by *in vitro* IF exposure on NSS cell-dependent depression was noticed at a ratio of 20:1 NSS/NK cells ($p<0.05$). A marked increase of the NSS cell suppressive effect occurred in the presence of PGE, at all NSS/NK cell ratios, as compared to the control mixtures. Pre-exposure to IF, followed by PGE incubation of NSS/effector cells did not prevent to a significant extent the PGE-induced NSS cell activation at all NSS/NK cell ratios.

DISCUSSION

It is generally accepted that antitumor activity of all interferons is due primarily to augmentation of the NK cell function as confirmed both by *in vivo* and *in vitro* experiments (1-7). However, some clinical trials do not find a relation between impaired interferon production and NK cell activity (4,15). Our data do not suggest a significant potentiation of the NK cell lytic ability related to *in vivo* beta-interferon administration, but such an effect is detected with mixed inoculation of interferon and the prostaglandin-inhibitor. These findings agree with other information demonstrating an inverse relationship between interferons and E-series prostaglandins in NK cell modulation (26). The *in vitro* exposure of control-group-derived NK cells to beta-interferon is followed by a significant increase of their cytotoxic abilities as revealed at 100:1, 50:1, and 25:1 E/T ratios, both when analyzed by cytotoxic

curves % and by lytic units 20%/10^7 of effector cells scored.

In regard to stimulation exerted by several interleukins on NK cell activity, the data are more discordant. Some workers note stimulation of natural cytotoxicity depending on T-cell growth factor (interleukin-2) (9,27) whereas others are not in agreement (7,10,11). Thus, with macrophage-derived soluble factors (e.g., interleukin-1) their adjuvanticity regarding several *in vivo* immune functions, including NK cell-mediated lysis, has been recognized in spite of other data suggesting that interleukin-like monokines suppress NK cell cytotoxicity under other special experimental conditions (8,12,13). On the other hand, there are other reports that confirm special interference between interferon and interleukin-dependent NK cell potentiation (6,7). Our experiments, carried out in mice, treated *in vivo* with interleukin-1 show a slight stimulation of natural cytotoxicity but only when a supplementary administration of indomethacin was applied at the highest E/T ratio. On the other hand, *in vitro* exposure to IL-1 of control-group-derived NK cells is followed also by a slight increase of the NK cell lysis level at the highest E/T ratios.

Prostaglandins, particularly those arising from the E-series, have consistently exerted a strong inhibition of NK cell anti-tumoral effects, that is supported both by *in vitro* exposure of NK cells to standard preparations of prostaglandins, and following *in vivo* administration of several prostglandin inhibitors (14,26). Our data also support a significant NK cell depressing effect exerted by a standard E_2-prostaglandin preparation *in vitro*, but no stimulation following *in vivo* indomethacin inoculation. Nevertheless, if a mixed IF/IND, or IL-1/IND treatement *in vivo* is applied, a "cumulative" augmentation of the natural cytotoxicity occurs, reflecting a probable association between some IF- or IL-1-dependent stimulating effects and a decrease in systemic prostaglandin. This observation may be taken together with other findings calling attention to potential antagonism between prostaglandins and interferons as well as between prostaglandins and interleukins (26,27).

By means of experimental procedures using suckling mice-derived "non-specific suppressor" cells *in vitro* mixed with control-group-derived NK cells and subsequently exposed to several immunomodulators, an evident increase of the NSS inhibiting activity in the presence of E_2-prostaglandin was noticed. On the other hand, interferon but not interleukin-1-*in vitro* treatment of NSS/NK cell mixtures was followed by diminution of NSS cell-induced NK cell inhibition only with the highest NSS/NK cell ratios. It is also noteworthy that pre-exposure to beta-interferon of NSS/effector cell mixtures did not prevent the prostaglandin-induced NSS cell activation. These data relate and supplement other findings describing the special significance given nowadays to the "suppressor/effector" cell balance in NK cell modulation (1,25).

REFERENCES

1. Saksela, E: Interferon and natural killer cells. In *Interferon*. Vol. 3. Gresser, I (Ed.). Acad. Press, London (1981), 45.
2. Ullberg, M, J Merril, M Jondal: Interferon-induced NK augmentation in humans: An analysis of target recognition, effector cell recruitment, and effector cell recycling. Scand. J. Immunol. 14 (1981), 285.
3. Lotzova, E, CA Savary, JU Gutterman, et al: Modulation of natural killer cell-mediated cytotoxicity by partially purified and cloned interferon-alpha. Cancer Res. 42 (1982), 2480.
4. Herberman, RB: Current status and perspective of natural killer cell research. Survey Synth. Pathol. Res. 1 (1983), 23.
5. Glaser, R, J Rice, CE Speicher, et al: Stress depresses interferon production by leukocytes concomitant with a decrease in NK cell activity. Behavioral Neuroscience 100 (1986), 675.
6. Joklik, WK: Interferons. In *Fundamental Virology*. Fields, BN, DM Knipe (Eds.). Raven Press, New York (1986), 281.
7. Kibler, R, MJ Hicks, AL Right, et al: A comparative analysis of cord blood and adult lymphocytes: Interleukin-2 and interferon production, NK cell activity, and lymphocyte populations. Diag. Immunol. 4 (1986), 201.

8. Dempsey, RA, CA Dinarelle, JW Mier, et al: The differential effects of human leukocyte/pyrogen/lymphocyte activating factor, T-cell growth factor, and interferon on human natural killer cell activity. J. Immunol. 129 (1982), 2504.
9. Grimm, E, A Mazunder, NZ Zhang, et al: Lymphokine-activated killer cell phenomenon. Lysis of natural killer resistant fresh solid tumor cells by interleukin-2 activated autologous human peripheral blood lymphocytes. J. Exp. Med. 155 (1982), 1823.
10. Ades, EW, A Hinson, LD Butler: Natural cytolytic activity in mice with natural or induced cellular defects. I. Differential ability of *in vitro* interleukin-2 addition to augment natural cytolytic function. Cell Immunol. 101 (1986), 15.
11. Brenner, BG, S Benarosu, RG Margolese: Peripheral blood NK cell activity in human breast cancer patients and its modulation by T-cell growth factor and autologous plasma. Cancer 58 (1986), 895.
12. Croll, AB, AG Morris: The regulation of human interferon gamma production by interleukin-1 and interleukin-2. Lymphokine Res. 5 (1986), Suppl., 115.
13. Endo, Y, K Matsushima, JJ Oppenheim: Mechanism of *in vitro* antitumor effects of interleukin-1. Immunobiol. 172 (1986), 316.
14. Medoe, ME, JJF Ogerr, JP Antel, et al: The modulation of human naturall killer cell activity by prostaglandins. J. Clin. Lab. Immunol. 7 (1982), 85.
15. Stoger, I, M Talas, M Benczur, et al: Lack of correlation between impaired interferon production and natural killer activity of lymphocytes in multiple sclerosis. Arch. Virol. 71 (1982), 259.
16. Voiculescu, C, S Nachtigal, N Gaicu, et al: A new mouse cell embryo line. Some aspects of the spontaneous *in vitro* transformation. Arch. Roum. Pathol. Exp. Microbiol. 34 (1975), 245.
17. Wietzerbim, J, S Stephanos, M Lucere: Physico-chemical characterization and partial purification of mouse immune interferon. J. Gen. Virol. 44 (1979), 773.
18. Rosenberg, SA, PE Lipsky: Macrophage-lymphocyte cooperation in human immune responses. In: *Cellular Interactions*. Elsevier North Holland Biomed. Press, Amsterdam (1981), 81.
19. Conlon, PJ: A rapid biologic assay for the detection of interleukin-1. J. Immunol. 131 (1983), 1280.
20. Itoh, K, R Suzuki, Y Umezu, et al: Studies of murine large granular lymphocytes. I. Tissue, strain, and age distribution of LGL and LAL. J. Immunol. 29 (1982), 395.
21. Stutman, O, EC Lattime: Natural cell-mediated cytotoxicity against tumors in mice: A heterogeneous system. Transplant Proc. 13 (1981), 752.
22. Ricardi, C, BM Vose, RB Herberman, et al: Modulation of interleukin-2-dependent growth of mouse natural killer cells by interferon and T lymphocytes. J. Immunol. 130 (1983), 228.
23. Wei Feng, C, A Wilson, R. Scollay, et al: Limit dilution assay and clonal expansion of all T cells capable of proliferation. J. Immunol. Methods 52 (1982), 307.
24. Timonen, T, CW Reynolds, JR Ortaldo, et al: Isolation of human and rat natural killer cells. J. Immunol Methods 51 (9182), 269.
25. Cudkewicz, G, PS Hochman: Regulation of natural killer activity by macrophage-like and other types of suppressor cells. In: *Developmental Immunology*. Grune and Stratton, New York (1979), 1.
26. Fligiel, HX, J Varani: Modulation of tumor cell motility by prostaglandins and inhibitors of prostaglandin synthesis. Exp. Cell Biol. 54 (1986), 128.
27. Svodersky, LP, HM Shepard, SA Spencer: Augmentation of human natural cell-mediated cytotoxicity by recombinant human interleukin-2. J. Immunol. 134 (1984), 714.

Dr. Constantin Voiculescu
Associate Professor
Faculty of Medicine Craiova
Petru Rares Street 4
Craiova 1100, RUMANIA