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Phagocytic Activity and Nitric Oxide Production of Circulating Polymorphonuclear Leukocytes from Patients with Peritoneal Carcinomatosis

Dejan Baskic, Ljubisa Acimovic, Aleksandar Djukic, Predrag Djurdjevic, Suzana Popovic, Biljana Milicic, Itana Labovic and Nebojsa N. Arsenijevic

From the Institute of Microbiology and Immunology (D. Baskic, S. Popovic, N.N. Arsenijevic), Clinic for Surgery (L. Acimovic), Institute of Pathophysiology, Faculty of Medicine, University of Kragujevac (A. Djukic, P. Djurdjevic), Department of Statistics, Faculty of Medicine, University of Kragujevac, Yugoslavia (B. Milicic), Public Health Institute, Podgorica, Yugoslavia (I. Labovic)

Correspondence to: Dr Dejan Baskic, Institute of Microbiology and Immunology, Faculty of Medicine, University of Kragujevac, 34000 Kragujevac, Yugoslavia. Tel: 381/34/331344. Fax: 381/34/331341. E-mail: baskic@medicus.medf.kg.ac.yu; baskic@eunet.yu

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Many studies have demonstrated an increase of neutrophils in patients with advanced cancer. However, the possible role of increased neutrophils in various neoplasms studied to date varies considerably. The authors examined the changes in white blood cell counts in patients with peritoneal carcinomatosis. Malonildialdehyde and nitric oxide (NO) plasma and ascitic fluid levels, phagocitic activity and the ability of the polymorphonuclear cells (PMNCs) to produce nitric oxide were also measured. An increase in PMNCs and decrease in lymphocytes was found in cancer patients. Compared with healthy controls, cancer PMNCs showed significant enhancement of phagocytosis. Similarly, pretreatment of healthy PMNCs with crude supernatants from short-term cultures of the peritoneal cells from ascitic fluid of patients with peritoneal carcinomatosis caused marked stimulation of PMNC phagocytosis. In addition, plasma and ascitic fluid nitric oxide levels in cancer patients were significantly higher than those found in control one. Most importantly, it was found that PMNCs from cancer patients release significantly more nitric oxide than corresponding normal controls. Therefore, considering the fact that neutrophils make up more than 50% of total leukocytes, these cells can play one of the most important roles in tumor biology.

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In cancer, numerous cellular and humoral elements of the immune system are activated and coordinately contribute to disease pathology. It has been known for a long time that mononuclear cells infiltrate tumor tissue. Although tumor-infiltrating mononuclear cells have been extensively studied over the past two decades, immunologists seeking new ways to treat cancer progression commonly overlook polymorphonuclear cells. However, it has been reported from clinical trials that an increase of leukocytes, mainly neutrophils, is noted in patients with advanced cancer (1). Moreover, some studies have shown that PMNC may infiltrate tumor tissue (2–4). Yet, neutrophils inhibited growth of one but stimulated growth of other tumors (5). Thus far, however, the roles of PMNC in the tumor-bearing host are still unclear.

MATERIAL AND METHODS

Patients

We analysed a group of 10 patients with peritoneal carcinomatosis, none of whom had received any chemo-, radio-, or endocrine therapy during the last three months. The control group consists of 10 healthy individuals and 10 stable patients on continuous ambulatory peritoneal dialysis. Abnormal peritoneal situations were excluded by blood cell count, peritoneal functional data, nocturnal peritoneal effluent cell population and bacterial culture.

Preparation of PMNC

Polymorphonuclear cells were isolated from heparinized venous blood using a density gradient (Lymphoprep 1.077,

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NYCOMED PHARMA AS, Oslo, Norway). PMNCs to be used in a bioassay were resuspended in supplemented media: RPMI 1640 (GIBCO Europe) contained 5% heat-inactivated autologous AB serum, 100 U/ml of penicillin (ICN Galenika, Zemun, Yu), 100 mg/ml streptomycin (ICN Galenika, Zemun, Yu) and 2 mM L-glutamine (Merck, Germany). Viability remained $>95\%$ by acridine orange/etidium bromide exclusion.

Phagocytosis assay

We used an assay developed by Vujanovic & Arsenijevic with minor modification (6). The isolated PMNCs were suspended in medium Haemacel (Jugoremedia, Zrenjanin, Yu) at a concentration of 1×10^6 cells/400 µl. The heat inactivated yeast particles, labeled with Neutral red (Merck, Germany) were then added at a 1:12 Effector Target ratio and cells were centrifuged at room temperature for 5 min at 50 g. Mixed suspension was incubated for 1h at 37°C. Non-ingested yeast particles were removed by washing twice with ice cold 0.02% EDTA. At least 300 cells were assessed per well, and each experiment used duplicate sample wells per condition. The average number of yeast particles ingested per one cell was defined as the phagocytic index (PI), whereas the percentage of cells ingesting at least one yeast particle was defined as the percent of phagocytosis (PP). Absolute phagocytic index (API) represents a number of yeast particles ingested per 100 cells ($API = PP \times PI$).

Nitrite determination

Nitric oxide synthesis was determined as the accumulation of nitrite. Nitrite concentrations in plasma (PL) and ascitic fluid (AF) were determined by Griess reaction following a procedure described by Green (8). Nitrite concentrations in cell free supernatants were measured by the Griess reaction according to the protocol of Stuehr & Nathan (7). Briefly, the isolated PMNC were adhered in 33 mm plastic dishes (Spectar, Cacak, Yu) at a concentration of 2×10^6 cells/ml. Cells were then cultured for 24 h at 37°C in 5% CO_2 in the supplemented media RPMI 1640. Nitrite concentration was determined in overnight culture supernatants. Values shown in the figures represent nanomoles of accumulated nitrite per 1.0 ml of the medium.

MDA determination

Lipid peroxidation product, malonildialdehyde (MDA), concentration in plasma and ascitic fluid were determined by the modified thiobarbituric acid assay of Ohkawa (9).

Cell culture

Peritoneal cells obtained from ascitic fluid of patients with peritoneal carcinomatosis were seeded in 33 mm plastic dishes. Cells were then cultured at 37°C in 5% CO_2 in the supplemented media RPMI 1640. Supernatants were harvested after 24 h, passed through 0.2 µm pore size filters and kept at -20°C until use. In some experiments dilutions of crude supernatants were used for immunomodulation of PMNCs as described.

Immunomodulation

PMNCs from control individuals were pretreated for 1 h with different concentrations (1/10, 1/1000, 1/10 000) of crude supernatants from short-term cultures of the peritoneal cells obtained from a cancer patient with peritoneal carcinomatosis, and then phagocytosis assay was performed.

Statistics

Statistical analyses were performed using commercially available software (SPSS version 8.0; SPSS Inc., Chicago, IL). Data are expressed as mean $\pm$SD. The distributions of data were evaluated for normality using the Kolmogorov-Smirnov test and than retested with a χ^2 test. Student's *t*-test was used for comparisons between two groups, for normally distributed parameters. For nonparametric variables, differences between two independent groups were determined by the Mann-Whitney U-test. Comparison between three and more groups of nonparametric variables was evaluated using Kruskal-Wallis test. A p-value < 0.05, from two-sided tests, was considered statistically significant.

The study was permitted by the Ethics Committee of the Medical School in Kragujevac.

RESULTS

White blood cell count

We examined the changes in the white blood cell count and their fractions in patients with peritoneal carcinomatosis. We found that cancer patients showed a normal count of total leukocytes, but an increased number of PMNCs (neutrophilia) (75.10 ± 11.87 vs. 62.70 ± 7.02; $p = 0.011$) accompanied by a decreased number of lymphocytes (lymphocytopenia) (17.10 ± 10.28 vs. 29.40 ± 7.66; $p = 0.007$). The mean number of monocytes was not significantly different between the cancer patients and the control group. The PMNC/MNC ratio was significantly higher in the cancer patients than in the normal controls (Fig. 1).

Phagocytosis

The phagocytic function of PMNCs was examined in 10 cancer patients. Compared with healthy controls, all tested parameters of PMNC phagocytosis were significantly enhanced in patients with peritoneal carcinomatosis. So, corresponding to PP (55.43 ± 10.44 vs. 42.86 ± 11.08; $p = 0.018$) (Fig. 2), PI and API, cancer patients' PMNCs were superior to those of control PMNC. According to this observation, we wanted to investigate whether observed differences are, in fact, due to tumor-derived products. As described in Materials and methods, immunomodulatory

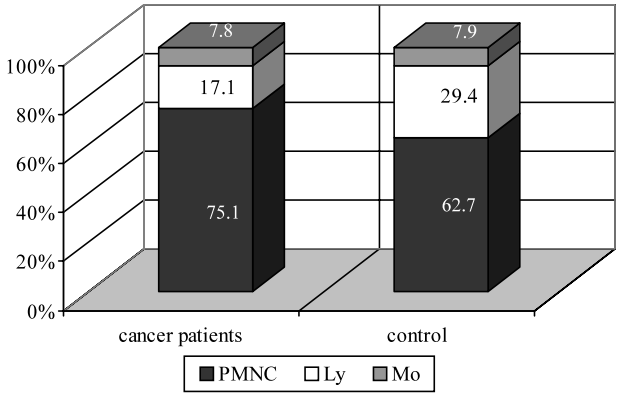

Fig. 1. The total and differential cell count. Cancer patients showed lymphocytopenia (17.10±10.28 vs. 29.40±7.66; p = 0.007) and neutrophilia (75.10±11.87 vs. 62.70±7.02; p = 0.011), but normal counts of monocytes and total leukocytes. Data are expressed as the mean of 10 different experiments.

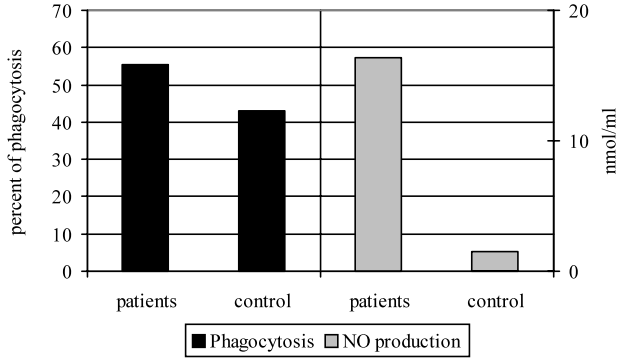

Fig. 2. Phagocytic activity and nitric oxide (NO) secretion by polymorphonuclear phagocytes. Percentage of phagocytosis was determined using a light microscope. Percentage of polymorphonuclear cells (PMNC) phagocytosis was significantly enhanced in patients with peritoneal carcinomatosis (55.43±10,44 vs. 42.86±11.08; p = 0.018). NO production was evaluated in cell supernatants as nitrite by Griss reaction. Cancer patients PMNC release more NO than corresponding normal controls (16.38±3.92 vs. 1.54±0.41; p = 0.016). Values represent the mean of duplicate samples from 10 different experiments.

assay was performed. It was found that only the highest tested concentration caused marked, statistically significant, stimulation of PMNC phagocytosis (1.33±0.48 vs. 1.00±0.00; p = 0.004), whereas this effect disappeared in the presence of a lower dose (Fig. 3).

Free radicals

We measured the intensity of lipid peroxidation (as a marker of peroxidation status) and nitrate levels (as an index of in vivo NO production) in plasma and ascitic fluid of patients with peritoneal carcinomatosis. Fig. 4 shows that plasma MDA levels in cancer patients were significantly

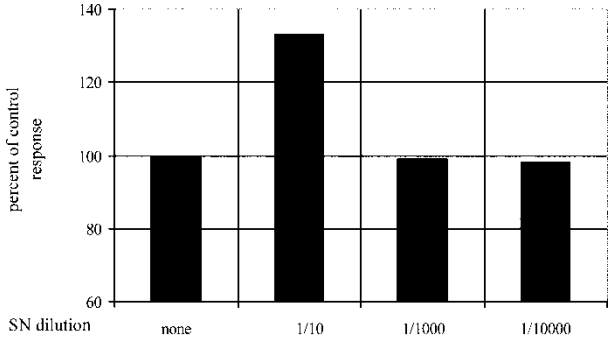

Fig. 3. Effects of crude supernatants on PMNC phagocytic activity. Polymorphonuclear phagocytes from healthy volunteers were pretreated with different concentrations of crude supernatants (Sn) from short-term cultures of the ascitic cells from patients with peritoneal carcinomatosis. Percentage of phagocytosis was evaluated using a light microscope. Highest tested concentration caused marked, statistically significant, stimulation of polymorphonuclear cells (PMNC) phagocytosis (1.33±0.48 vs. 1.00±0.00; p = 0.004). Results obtained were presented as the percentage of control response. Data are expressed as the mean of 10 experiments.

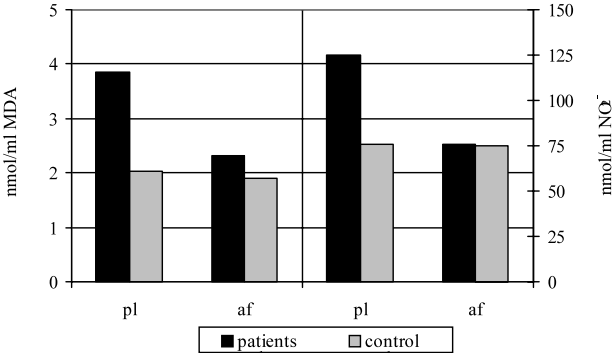

Fig. 4. Plasma and ascitic fluid malonildialdehyde (MDA) and nitrite levels. MDA concentrations in plasma (pl) and ascitic fluid (af) were determined by thiobarbituric acid assay. Nitrite concentrations in plasma (pl) and ascitic fluid (af) were determined by Griess reaction. Elevated plasma MDA (3.85±0.36 vs. 2.03±0.29; p = 0.007) and nitrite (112.00±26.97 vs. 76.83±11.83; p = 0.038) levels were observed among cancer patients with peritoneal carcinomatosis. Values represent the mean of duplicate samples from 10 different experiments.

higher than those found in control one (3.85±0.36 vs. 2.03±0.29; p = 0.007). However, no difference was found in the ascitic fluid MDA levels (2.33±0.21 vs 1.98±0.18; p = 0.487). Next, we have found that patients with peritoneal carcinomatosis have elevated plasma (112.00±26.97 vs. 76.83±11.83; p = 0.038), but not ascitic fluid (76.30±26.97 vs. 75.52±18.10; p = 0.230) nitrate levels (Fig. 4). In addition, we investigated the potential of polymorphonuclear phagocytes from cancer patients to produce NO. We have shown that cancer patients' PMNCs release more NO than corresponding normal controls (16.38±3.92 vs. 1.54±0.41) and the statistical significance was p = 0.016 (see Fig. 2).

DISCUSSION

Polymorphonuclear neutrophils, as phagocytes, play the crucial role in the host defense. The first description of phagocytosis in the late nineteenth century, Metchnikoff's classic experiment, established a conceptual link between the engulfment of senescent or apoptotic cells (as a part of normal development) and the host defense. This work has defined macrophages and polymorphonuclear neutrophils as gatekeepers in the first-line host defense. During past years the predominant attention has been paid to the role of phagocytes, especially neutrophils, in the host defense against infection. Yet, only recently more attention has been paid to PMNCs and phagocytosis in cancer. Experiments in the 1970s and 1980s, as well as some recent studies, have shown that most of the functions of PMNC in patients with advanced cancer were impaired or normal when compared with healthy individuals (10). These data supported the hypothesis that depressed granulocyte function may contribute to an increased susceptibility to infection and may be considered as an additional factor that favors tumor dissemination.

In this study, we have found that cancer patients showed lymphocytopenia and neutrophilia, but normal count of monocytes and total leukocytes. Similar results have already been reported from other laboratories (1, 11, 12). Our earlier studies have shown that macrophages and lymphocytes are the main components of the ascitic fluid. Polymorphonuclears were rarely seen (13, 14). However, contrary to most other (15–17), but not all (18, 19), studies, we have found that peripheral blood PMNCs from cancer patients showed significantly higher phagocytic activity and impressive increase in NO production, compared with the controls. Moreover, we observed an elevation in plasma nitrate and MDA levels among cancer patients. Several papers have also demonstrated that plasma concentration of nitrate/nitrite was higher in patients with metastatic cancer compared with normal subjects (20–22).

As a cause for the increased phagocytosis and ability to produce NO, one may speculate whether the increase of neutrophils may represent an in vivo antitumor defense activity. A lot of studies have demonstrated that numerous PMNCs accumulate in the cancer region (2–4, 23). Infiltration of tumor by immune cells requires the local production of chemoattractants. Tumor cells generate some of the most powerful neutrophil chemoattractants, such as IL-8 (24). Curiously, many of chemoattractants, such as LTB4, IL-8, growth-related oncogene alpha (GRO-alpha) and TGF-beta, can be secreted by activated PMNC in an autocrine loop. Chemokine (25) and cytokine (26) gene transfection into tumor cells, as well as intralesional (27) and systemic (28) cytokine therapy, retard tumor growth. This growth attenuation was correlated with neutrophil and monocyte infiltration (29). Moreover, Pericle et al. demonstrated direct killing of IL-2 transfected tumor cells by human neutrophils (30).

There are two possible mechanisms for how activated PMNCs can kill neoplastic cells and inhibit tumor growth. First, PMNC possess a range of potent proteinases and hydrolases, as well as the ability to generate a series of reactive oxygen and nitrogen intermediates, all of them having the potential for inhibiting tumor growth. For example, Uchida et al. have shown that tumor cell apoptosis by rIFN-gama activated neutrophils is mediated by L-arginine-deriveded nitrogen oxidation products (31). In addition, recombinant, fully human IgA1, Fc alpha RI or Fc gamma RI-directed antitumor bispecific antibody mediated specific lysis of appropriate tumor antigen-expressing target cells with purified PMNCs (32). Consistently with these findings, it should be noted that successful cytokine immunotherapy is often accompanied by an increase in the number and function of PMNCs (33). Similarly, following chemotherapy, both stimulated and unstimulated PMNCs generate the increased amounts of superoxide anion and hydrogen peroxide, which are accompanied by an increased formation of lipid peroxidation products, such as MDA. Therefore, many anti-cancer drugs can, in vivo, augment reactive oxygen/nitrogen species generation and lipid peroxidation and thus, cause tumor cell lysis (34). Second, indirect mechanisms may involve the production of downstream mediators by intratumoral PMNC (e.g. IL-1 beta, IL-12, TNF-alpha) leading to T-cell and macrophage recruitment and activation.

In contrast, it has been stated that neutrophils may promote transformation, growth, progress and metastasis of cancer. Several papers have demonstrated that nitric oxide, a small diatomic molecule, in the presence of superoxide anion, generates reactive intermediates which may modify DNA bases, inactivate DNA repair enzymes and thus contribute to genotoxicity and mutagenesis (35); that inflammatory neutrophils may represent an important metabolic source of endogenous NO and carcinogenic nitrosamines at sites of chronic inflammation (36); that inducible nitric oxide synthase and reactive nitrogen intermediates, including peroxinitrite, are produced (among others) specifically by tumor-infiltrating neutrophils during the tumor promotion (37); and that tumor infiltrating neutrophils, as a potential source of this genotoxic species, may be mutagenic and contribute to the burden of genetic abnormalities associated with tumor progression (38).

Generally, this evidence indicates that the PMNCs should not be ignored when considering the molecular and cellular processes that regulate tumor progression/regression and should feature in the search for the new diagnostic and therapeutic procedures in cancer patients' treatment.

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REFERENCES

1. Satomi A, Murakami S, Ishida K, Mastuki M, Hashimoto T, Sonoda M. Significance of increased neutrophils in patients with advanced colorectal cancer. Acta Oncol 1995; 34: 69–73.
2. Fossati G, Ricevuti G, Edwards SW, Walker C, Dalton A, Rossi ML. Neutrophil infiltration into human gliomas. Acta Neuropathol (Berl) 1999; 98: 349–54.
3. Potapov IuN, Krutova TV, Khaleev DV, Pashkov VS. The intensity of intratumor infiltration with polymorphonuclear neutrophils in different stages of the development of Lewis' carcinoma. The effect of the surgical removal of the primary node. Izv Akad Nauk Ser Biol 1994; 1: 164–6.
4. Morioka T, Baba T, Black KL, Streit WJ. Inflammatory cell infiltrates vary in experimental primary and metastasic brain tumors. Neurosurgery 1992; 30: 891–6.
5. Chen RL, Reynolds CP, Seeger RC. Neutrophils are cytotoxic and growth inhibiting for neuroblastoma cells with an anti-GD2 antibody, but without cytotoxicity can be growth stimulating. J Cancer Immunol Immunother 2000; 48: 3–12.
6. Vujanovic NL, Arsenijevic NN, Vlajic M. Modulation of polymorphonuclear neutrophil motility and monocyte phagocytosis by factor released from human primary breast cancer tissue. Period Biol 1986; 88(Suppl 1): 53–5.
7. Stuehr DJ, Nathan CF. Nitric oxide: a macrophage product responsible for cytostasis and respiratory inhibition in target tumor cells. J Exp Med 1989; 169: 1543–55.
8. Green LC, Wagner DA, Glogowski J, Skipper PJ, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and (^{15}N) nitrate in biological fluids. Anal Biochem 1982; 126: 131–9.
9. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351–8.
10. Wiezer MJ, Meijer C, Wallast-Groenewoud HP, et al. Impaired leukocyte phagocytosis in patients undergoing hemihepatectomy for liver metastases. Liver Transpl Surg 1999; 5: 238–45.
11. Ietomi K. A study on the role of granulocytes in carcinoma bearing hosts: G/L ratio as a new host indicator. J Jpn Soc Cancer Ther 1990; 25: 662–71.
12. Garcia-Gonzalez JE, Rojas-Espinosa O, Aguilar-Santelises M. Phagocytic activity of circulating polymorphonuclear leukocytes from patients with carcinoma of the uterine cervix. Rev Latinoam Microbiol 1992; 34: 135–41.
13. Baskic D, Acimovic Lj, Samardzic G, Vujanovic NL, Arsenijevic NN. Blood monocytes and tumor-associated macrophages in human cancer: differences in activation levels. Neoplasma 2001; 48: 169–74.
14. Baskic D, Acimovic Lj, Samardzic G, Djurdjevic P, Djukic A, Arsenijevic NN. The altered activation state of macrophages isolated from ascitic fluid of patients with peritoneal carcinomatosis. Arch Oncol 2000; 8: 99–103.
15. Amati L, Caradonna L, Greco B, Leo S, Caccavo D, Jinillo E. Impairment of phagocytic and T-cell-mediated antibacterial activity and plasma endotoxins in patients with untreated gastrointestinal cancer. Scand J Gastroenterol 1998; 33: 847–52.
16. Hofmann WK, Stauch M, Hoffken K. Impaired granulocytic function in patients with acute leukemia: only partial normalization after successful remission-inducing treatment. Cancer Res Clin Oncol 1998; 124: 113–6.
17. Arii K, Tanimura H, Iwahashi M, et al. Neutrophil functions and cytokine production in patients with gastric cancer. Hepatogastroenterology 2000; 47: 291–7.
18. Szucs S, Kavai M, Varga C, et al. Changes in superoxide anion production and phagocytosis by circulating neutrophils during tumor progression in a rat model. J Cell Immunol 1996; 170: 202–11.
19. Shinomiya N, Tsuru S, Tsugita M, Takemura T, Katsura Y, Nomoto K. Differential function of polymorphonuclear leukocytes between in vivo and in vitro in tumor-bearing mice. Clin Lab Immunol 1990; 33: 61–8.
20. Timoshenko AV, Dubovskaya LV, Karvatskaya OD, Zharkov VV, Andre S, Gabius HJ. NO-dependent regulation of lectin- and menadione-induced H2O2 production by cells from pleural effusions of lung cancer patients and by immune cells. Int J Onkol 1999; 14: 793–8.
21. Miles D, Thomsen L, Balkwill F, Thavasu P, Moncada S. Association between biosynthesis of nitric oxide and changes in immunological and vascular parameters in patients treated with interleukin-2. Eur J Clin Invest 1994; 24: 287–90.
22. Moriyama A, Masumoto A, Nanri H, et al. High plasma concentrations of nitrite/nitrate in patients with hepatocellular carcinoma. Am J Gastroenterol 1997; 92(9): 1520–3.
23. Thomas C, Nijenhuis AM, Timens W, Kuppen PJ, Daemen T, Scherphof GL. Liver metastasis model of colon cancer in the rat: immunohistochemical characterization. Invasion Metastasis 1993; 13: 102–12.
24. Bellocq A, Antoine M, Flahault J, et al. Neutrophil alveolitis in bronchioloalveolar carcinoma: induction by tumor-derived interleukin-8 and relation to clinical outcome. Am J Path 1998; 152: 83–92.
25. Giovarelli M, Cappello P, Forni G, et al. Tumor rejection and immune memory elicited by locally released LEC chemokine are associated with an impressive recruitment of APCs, lymphocytes and granulocytes. J Immunol 2000; 164: 3200–6.
26. Zilocchi C, Stoppacciaro A, Chiodoni C, Parenza M, Terrazzini N, Colombo MP. Interferon gamma-independent rejection of interleukin 12-transduced carcinoma cells requires CD4+ T cells and granulocyte/macrophage colony-stimulating factor. J Exp Med 1998; 188: 133–43.
27. Davidson JA, Musk AW, Wood BR, et al. Intralesional cytokine therapy in cancer: a pilot study of GM-CSF infusion in mesothelioma. J Immunother 1998; 21: 389–98.
28. Shetye J, Ragnhammar P, Liljefors M, et al. Immunopathology of metastases in patients of colorectal carcinoma treated with monoclonal antibody 17-1A and granulocyte macrophage colony-stimulating factor. Clin Cancer Res 1998; 4: 1921–9.
29. Lee LF, Hellendall PR, Wang Y, et al. IL-8 reduced tumorigenicity of human ovarian cancer in vivo due to neutrophil infiltration. J Immunol 2000; 164: 2769–75.
30. Pericle F, Kirken RA, Epling-Burnette PK, Blanchard DK, Djeu JY. Direct killing of interleukin-2-transfected tumor cells by human neutrophils. Int J Cancer 1996; 66: 367–73.
31. Uchida T, Yamashita T, Araki A, Watanabe H, Sendo F. IFN-gamma-activated rat neutrophils induce tumor cell apoptosis by nitric oxide. Int J Cancer 1997; 71: 231–6.
32. Huls G, Heijnen IA, Cuomo E, et al. Antitumor immune effector mechanism recruited y phage display-derived fully human IgG1 and IgA1 monoclonal antibodies. Cancer Res 1999; 59: 5778–84.
33. Galati G, Rovere P, Citterio G, et al. In vivo administration of GM-CSF promotes the clearance of apoptotic cells: effects on monocytes and polymorphonuclear leukocytes. J Leukoc Biol 2000; 67: 174–82.

34. Sangeetha P, Das UN, Koratkar R, Suryaprabha P. Increase in free radical generation and lipid peroxidation following chemotherapy in patients with cancer. Free Radic Biol Med 1990; 8: 15–9.
35. Wink DA, Laval J. The Fpg protein, a DNA repair enzyme, is inhibited by the biomediator nitric oxide in vitro and in vivo. Carcinogenesis 1994; 15: 2125–9.
36. Byun J, Henderson JP, Mueller DM, Heinecke JW. 8-Nitro-2′-deoxyguanosine, a specific marker of oxidation by reactive nitrogen species, is generated by the myeloperoxidase-hydrogen peroxide-nitrite system of activated human phagocytes. Biochemistry 1999; 38: 2590–600.
37. Haqqani AS, Kelly JF, Birnboim HC. Selective nitration of histone tyrosine residues in vivo in mutatect tumors. J Biol Chem 2002; 277: 3614–21.
38. Sandhu JK, Privora HF, Wenckebach G, Birnboim HC. Neutrophils, nitric oxide synthase, and mutations in the mutatect murine tumor model. Am J Pathol 2000; 156: 509–18.