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Original scientific paper

Cross-talk between NKT and Regulatory T cells (Tregs) in Modulation of Immune Response in Patients with Colorectal Cancer following Different Pain Management Techniques

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A B S T R A C T

Natural killer T (NKT) and regulatory T cells (Tregs) play an important role in innate immune response. Natural killer (NK) and NKT cells are indispensable factors in the body's ongoing defense against tumor development, as well as viral infection. NKT cells are a subset of T cells that shares properties of natural killer cells and conventional T cells. They are involved in innate immune responses, tumor rejection, post transplantation immunotherapy, immune surveillance and control of autoimmune diseases. They may also play both protective and harmful roles in the progression of certain autoimmune diseases, such as diabetes, lupus, atherosclerosis, and allergen-induced asthma. Immune surveillance involves the process whereby precancerous and malignant cells are recognized by the host immune system as damaged and are consequently targeted for elimination. The pharmacological management of postoperative pain in patients with malignancies uses very different techniques whose possible cytotoxic functions we still known very poor. The present study compared effects of two different postoperative pain management techniques in patients undergoing colorectal cancer surgery on the innate immunity. Our data indicate that the patients with colorectal cancer have significantly increased the percentage of Tregs and NKT cells. The values were statistically higher during epidural analgesia in comparison with intravenous analgesia, indicating that epidural pain management technique ameliorate the immune suppression after surgery.

Key words: *colorectal cancer, epidural analgesia, innate immunity, intravenous analgesia, NKT cells, regulatory T cells.*

Introduction

The human response to malignancies, surgical or chronic stress is linked to alteration in immune function, observed as decreases in natural killer (NK) cell cytotoxicity, lower antibody titers and decreased lymphocyte proliferation[1]. Anesthesia and analgesia, associated with surgical stress suppress the immune response following different complex mechanisms: direct effect on the immune system and/or by activating the hypothalamic-pituitary-adrenal axis and sympathetic nervous system[2]. During stress (surgery, hypo-and hyperthermia, hyperglycemia, blood transfusion, postoperative pain), anesthetics suppress immunity in the perioperative period due to its direct suppressive effect on cellular and humoral immunity, and affecting the function of immunocompetent cells, the expression and secretion of inflammatory mediated genes[3]. Tumor cells provide antigenic stimulation of T cells and interact with the tumor-infiltrated innate immune cells secreting cytokines that are crucial for T-cell differentiation. Virgin CD4 + T cells can be differentiated into different subsets of CD4 + T cells, including Th1, Th2, regulatory T cells (Tregs) and IL-17-T cells (Th17), depending on the strength of anti-

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genic stimulation and cytokines milieu. Alternatively, the natural CD4 + CD25 + Treg cells directly derived from the thymus may contribute to cross-reaction with some antigens expressed on cancer cells, thus promoting its spread and accumulation in the tumor microenvironment[4]. A large number of studies have described the direct role of Tregs in, if not immediately at the beginning of tumor growth, at least in the prevention of immunity in a well-described tumors[5]. Thereby, Tregs as a subset of T cells represent a unique subpopulation which may affect on development and prognosis of many different ilnesses: alergic diseases[6], infection[7], malignancies[8], autoimmunity[9], graft-versus host disease[10] and environmental diseases[11]. On the other hand NKT cells are a type of T cells that share the common features of natural killer cells (NK) and conventional T cells. NKT cells are involved in the regulation of innate immune responses, tumor rejection in the posttransplantation immunotherapy, immunologic survival, control of autoimmune disease (diabetes type I, multiple sclerosis, SLE, myasthenia gravis, asthma, RA, etc.), the number of pathological conditions in which regulate viral infection in vivo and tumor growth. One can play assertive, but also detrimental role in the progression of certain autoimmune diseases such as diabetes, lupus, atherosclerosis and asthma[12–15]. Nowdays, we distinguish NKT cells type I and type II. They are differing according to: phenotype characteristics (CD4 + or double negative (CD4-CD8-), distribution in different tissues and NK 1.1 expression. The protective NKT cells are type I NKT cells, which are double negative, mature in the liver and possibly NK 1.1 positive and produce IFN-γ, which mediates protective antitumor role. Suppressive NKT cells are type II NKT cells, CD4 +, react to sulfatid ion and through IL-13 suppress the immune response to tumors[16]. Assumptions suggest that NKT cells can act as helper cells for Treg, thus represent a link between two natural populations of regulatory T cells. The aim of this study was to investigate the characteristics of human regulatory T and NKT cells of patients with colorectal cancer, during intravenous and epidural analgesia.

Subjects and Methods

Our study includes 80 patients with colorectal cancer. We are taking venous blood from the periphery (5 ml) prior to the start of analgesia, i.e. before surgery and the 1st and 6th postoperative day during the implementation of epidural or intravenous analgesia. Peripheral blood leukocytes (PBL) were isolated and using flow cytometry technique (FACSCalibur) are determined individual lymphocyte subpopulations: T, B lymphocytes, NKT cells (CD3+ CD56+) and T-regulatory cells (CD4 + CD25 + FoxP3 +). Statistical analysis was done in computer program Statistica 7.1 (StatSoft, Inc., Tulsa, OK, USA). Graphs and tables were done in computer program Microsoft Excel.

Results

In figure 1 are shown the changes in phenotype of patients with colorectal cancer during six postoperative days.

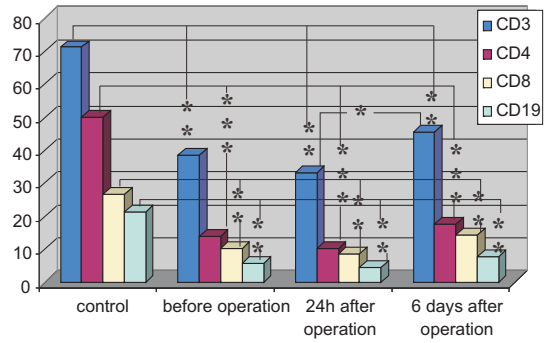

Fig. 1. The dinamic changes in phenotype of patients with colorectal cancer during six postoperative days (=p<0,05; **=p<0,01; ***p<0,001).*

The percentage of total T lymphocytes (CD3+ cells) are statistically significantly diminished in patients with colorectal cancer before the operation, as well as during six postoperative days, but interestinglly, the 6th postoperative day, the values were higher than in the 1st day, indicating that in the recovery period CD3+ are the first subpopulation which is started to emerge. All other subpopulations (T helpers-CD4+ cells, T cytotoxic cells-CD8+ and B lymphocytes-CD19+) remain lower during first six days. NK (CD3-CD56+ cells) and NKT (CD3+ CD56* cells) subpopulations were significantly lower in patients undergoing intravenous analgesia than in patients in which used epidural pain management technique (Figure 2).

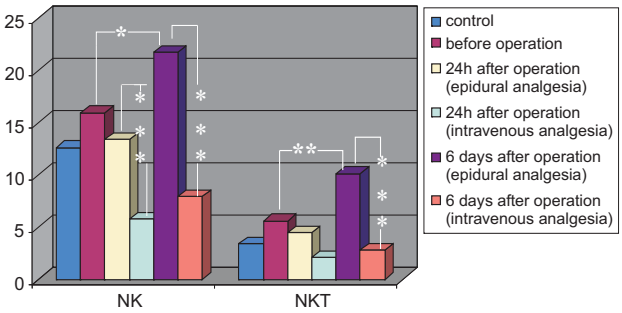

Fig. 2. The frequency of changes of NK and NKT cells during epidural/intravenous analgesia.

During analgestic period of six days only in patients with epidural analgesia we have noticed augmentation of NK and NKT cells.

Similar situation we have seen in the proportion of regulatory T cells (CD3+CD25+FoxP3+). Patients with colorectal cancer have statistically higher proportion of Tregs than control group. In group with intravenous an-

algesia at the 1st, as well at the 6th postoperative day, this values very lower in comparison with the patients with epidural analgesia (Figure 3).

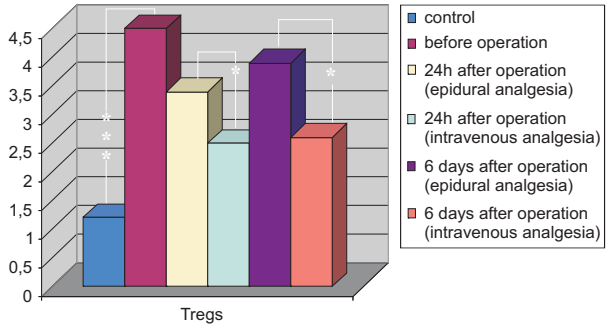

Fig. 3. The changes in percentage of Tregs cells in patients with colorectal cancer during epidural/intravenous analgesia.

Our data indicate that the patients with colorectal cancer have significantly increased the percentage of NK, NKT and Treg cells, which may interplay with the number and function of other lymphocytes subpopulation, as well as antigen presenting cells (APC), especially dendritic cells.

Discusion and conclusion

Tregs and NKT share common characteristics in their ability to regulate the immune response: both cells can suppress cytokine production and proliferation in target tissues. The percentage of these cells of innate immunity were higher in patients undergoing epidural analgesia, which may interplay with the function of innate immune response during recovery period after surgical operation and their values might be used as a predictor marker. According to the fact that both of these subpopulation were higher in patients with colorectal cancer, we can think about NKT and Tregs-based immunotherapy or vaccine. Despite enormous advancements in tumor immunology over the last two decades, the cascade of molecular events leading to immune-mediated tumor rejection is still incompletely understood and clinical results achieved with cancer vaccines are limited[17]. Nowdays, many recent studies tend to focus on three main areas that are of particular interest for the development of new vaccination strategies: (a) cellular molecular mechanisms of immune tolerance to malignant cells, (b) synergism between the innate and acquired immune responses, and (c) interaction tumors and the immune system within the tumor microenvironment[17]. The appropriate postoperative pain management by epidural analgesia can modulate cell mediated immunity of these patients after colorectal surgery. These proportions depend of the status of illness and distinguish between the first and the 6th postoperative day. The data emphasize that during the postoperative pain management, epidural analgesia prevents some immune dysfunctions, contributing to the more efficient postoperative recovery than the patients undergoing intravenous analgesia.

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REFERENCES

1. SEGERSTROM SC, MILLER GE, Psychol Bull, 130 (2004) 601. — 2. KUROSAWA S, KATO M, J Anesth 22, (2008) 263. — 3. KELBEL I, WEISS M, Curr Opin Anaesthesiol, 14 (2001) 685. — 4. WANG HY, WANG R-F, Current Opinion in Immunology, 19 (2007) 217. — 5. ZOU W, Nat Rev Immunol 6 (2006) 295. — 6. XYSTRAKIS E, URRY Z, HAWRYLOWICZ CM, Curr Opin Allergy Clin Immunol, 7 (2007) 535. — 7. SUTMULLER RP, MORGAN ME, NETEA MG, GRAUER O, ADEMA GJ, Trends Immunol, 27 (2006) 387. — 8. PIERSMAA SJ, WELTERSB MJP, VAN DER BURGA SH, Human Immunology, 69 (2008) 241. — 9. SAKAGUCHI S, SAKAGUCHI N, SHIMIZU J, YAMAZAKI S, SAKIHAMA T, ITOH M, KUNIYASU Y, NOMURA T, TODA M, TAKAHASHI T, Immunol Rev, 182 (2001) 18. — 10. TAWARA I, SHLOMCHIK WD, JONES A, ZOU W, NIEVES E, LIU C, TOUBAI T, DURAN-STRUUCK R, SUN Y, CLOUTHIER SG, EVERS R, LOWLER KP, LEVY RB, REDDY P, J Immunol, 185 (2010) 3866. — 11. MIĆOVIĆ V, VOJNIKOVIĆ B, BULOG A, ČOKLO M, MALATESTINIĆ Đ, MRAKOVČIĆ-ŠUTIĆ I, Coll. Antropol, 33 (2009) 743. — 12. MOLLING JW, KO¨LGEN W, VAN DER VLIET HJJ, BOOMSMA MF, KRUIZENGA H, SMORENBURG CH, MOLENKAMP BG, LANGENDIJK JA, LEEMANS CR, VON BLOMBERG BME, SCHEPER RJ VAN DEN EERTWEGH AJM, Int J Cancer, 116 (2005) 87. — 13. BRAUNER H, ELEMANS M, LEMOS S, BROBERGER C, HOLMBERG D, FLODSTRÖM-TULLBERG M, KÄRRE K, HÖGLUND P, J Immunol, 184 (2010) 2272. — 14. GABRIEL L, MORLEY BJ, ROGERS NJ, Ann N Y Acad Sci, 1173 (2009) 435. — 15. BJÖRKBACKA H, NILSSON J, J Innate Immun, 2 (2010) 305. — 16. BERZOFSKY J.A. AND TERABE M, J Immunol, 180 (2008) 3627. — 17. MOCELLIN S, ROSSI CR, NITTI D, Experimental Cell Research 299 (2004) 267.

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MEĐUDJELOVANJE NKT I REGULACIJSKIH T STANICA (TREGS) PRI MODULACIJI IMUNOLOŠKOG ODGOVORA U BOLESNIKA S RAKOM DEBELOG CRIJEVA TIJEKOM RAZLIČITIH ANALGETSKIH TEHNIKAMA

S A Ž E T A K

NKT i regulacijske T stanice (Tregs) igraju važnu ulogu u urođenom imunološkom odgovoru. Stanice prirodne ubojice (NK) i NKT stanice su neophodni čimbenici u tijelu tijekom obrane od razvoja tumora, kao i tijekom virusne infekcije. NKT stanice su subpopulacija T stanica koja dijeli svojstva prirodnih stanica ubojica i konvencionalnih T stanica. Uključene su u urođeni imunološki odgovor, tumorsko odbijanje, u imunoterapiji nakon transplantacije, imunološkom nadzoru i kontrolu autoimunih bolesti. One također mogu igrati i zaštitnu, ali i štetnu ulogu u napredovanju određenih autoimunih bolesti, kao što su dijabetes, lupus, ateroskleroza, te alergenom-uzrokovana astma. Imunološki nadzor uključuje proces u kojem prekancerozne i maligne stanice bivaju prepoznate od strane domaćina kao štetne te imunološki sustav teži njihovom uklanjanju. Citotoksični učinak farmakoloških tehnika ublažavanja poslijeoperacijske boli u bolesnika s malignih bolestima je još uvijek slabo poznat. U ovom istraživanju uspoređivali smo učinke dviju različitih poslijeoperacijskih tehnika suzbijanja boli u bolesnika podvrgnutih operaciji karcinoma debelog crijeva. Naši podaci pokazuju da pacijenti s karcinomom debelog crijeva imaju znatno povećani postotak Tregs i NKT stanice. Vrijednosti su bile statistički značajno viša tijekom epiduralne analgezije u usporedbi s intravenskom analgezijom, što znači da epiduralna tehnika suzbijanja boli poboljšava imunosupresiju nastalu tijekom operativnog zahvata.