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Indices of immune activation after bronchial carcinoma surgery

Disfunction of the immune system in neoplasmatic diseases is additionally modulated by chemo-and radiotherapy as well as by surgical procedures.The antineoplastic immune response consists of antibody production and cell mechanisms involving the activity of cytotoxic Tc-lymphocytes, NK cells, cytokines secreted by Th-lymphocytes and activated macrophages (10, 14, 20).The Th-lymphocyte activation is mediated by the following cytokine profile (11): Th1 regulating the cell response by the synthesis of IL-2 , IFN-γ, TNFα, IL-1, and Th2 assisting in the synthesis of specific antibodies by B-lymphocytes with IL-4, IL-5 and IL-6.

The key role of IL-2 concerns the activation of NK cells and T-lymphocytes, which induces the LAK (lymphocyte activated killer) activity of some of them. The LAK cells found in neoplastic infiltration show cytotoxic properties and are characterized in vitro by significantly higher antineoplastic activity than NK cells or T-lymphocytes (12). The activation of appropriate cells by IL-2 is regulated by expression of the surface receptors for IL-2R and by the presence of the soluble form of receptors, sIL-2R. The soluble receptor for IL-2 is a relevant factor regulating the action of this cytokine. It may bind IL-2 in the plasma preventing its interactions with the receptor on the cell surface, which is likely to lead to a substantial decrease in the physiological function of T and NK cells (17).The activation of Th2 cells, the synthesis of a suitable cytokine profile, including IL-6, is connected with the synthesis of antibodies against neoplastic antigens and with the activation of acute phase proteins (16). The acute-phase reaction is an early nonspecific response of the organism to the action of such factors as tissue injury, including operative procedures, tissue infection and necrosis caused by acute ischaemia or neoplasm.

The importance of the immunomodulating role of CRP results from its abilities to modify the effector cells, its chemotactic effects on the multinuclear leukocytes, induction of superoxide production by these cells and activation of the complement system (2).

Serum amyloid A (SAA), the protein which synthesis is induced by IL-1 d IL-6 is capable of modulating high density lipoprotein (HDL) metabolism and is involved in the repair processes by the collagen induction and by negative feedback between SAA proteins and immunoregulating cytokines (15). Determinations of the acute phase are used as sensitive markers of the severity of infections-inflammatory processes, acute trauma and transplant rejection.

The studies of the levels of acute phase proteins such as CRP and SAA conducted so far on patients with neoplastic diseases showed the serum concentration changes and some correlation

between their concentration and neoplastic stages (3). The cytokine levels of their soluble receptors and acute phase proteins are the indices of activation of certain lymphocyte subpopulation and appropriate cells. The changes in the above-mentioned parameters are found in patients with neoplastic diseases and are further modified towards immunosuppression as a result of trauma during surgery.

The aim of the study was to examine the levels of IL-2 and the soluble receptor, sIL-2R involved in the activation of Th1 subpopulation and C-reactive protein (CRP) and serum amyloid A (SAA); the protein whose synthesis is induced by IL-6 and IL-1 in patients operated on for bronchial carcinoma.

MATERIAL AND METHODS

The studied group consisted of 35 patients operated on for bronchial carcinoma. The immune parameters were examined before, and three, seven, 10 days after the operation. Control group 1 consisted of 17 patients requiring thoracic surgery due to other reasons-tuberculosis with its complications (aspergilloma) in eight cases, benign tumor of the lung or mediastinum in three cases, and emphysematous changes in two cases. The remaining indications for surgery included: malformation of the lung, funnel-shaped thorax, bronchiectasis. The immune parameters were examined before, and three, seven, 10 days after the surgical procedure. Control group 2 included 22 healthy individuals whose parameters were examined only once. During examinations and at least two months prior to them the patients did not show any symptoms of infection and did not take drugs which might affect the immune parameters.

The blood samples were collected from the ulnar vein to the test-tubes containing EDTA and to the dry test-tubes for clotting. The material was centrifuged, the plasma and serum were frozen at -80°C. ELISA method was used to determine IL-2 and sIL-2R levels according to the procedure recommended by the producers. The following kits were used: for IL-2-Enologen Human Interleukin-2 ELISA (Endogen, Inc. USA). The test sensitivity < 6 pg/ml; for sIL-2R-CELLFREE Interleukin-2 receptor Kit (T Cell Diagnostic, Inc. USA). The test sensitivity was about 24 U/ml. The levels of CRP were determined by using the Manchini radial immunodiffusion method. The Beringer werke AG plates were used. The levels of SAA proteins were examined by the ELISA method using Cytoscreen Tm Immunoassay Kits (Biosource International).

RESULTS

Analysis of the serum IL-2 levels in the patients and controls revealed that in the patients with bronchial carcinoma the concentration of this cytokine was three times higher than the one whih was observed in the healthy controls (Table 1). The mean concentration of the soluble receptor for IL-2 was also found to be statistically significantly higher in the group of patients (Tab. 2). The IL- 2 values observed three days after surgery showed a decrease of IL-2 in the patients with bronchial carcinoma and those subjected to surgery due to other reasons. The examinations carried out seven and 10 days after the procedure showed a systematic increase of IL-2. In patients with bronchial carcinoma the IL-2 values 10 days after surgery were higher than those observed before the operation. Three days after surgical procedures the sIL-2 levels significantly increased in both patient groups, however, during the next examinations the changes were different in those groups: sIL-2R decreased in the patients with bronchial carcinoma, while its further increase was observed in the remaining patients.

Table 1. Serum IL-2 levels in the individual groups before and after surgery

Study group	IL-2 level before surgery pg/ml	IL-2 level three days after surgery	IL- 2 level seven days after surgery	IL-2 level 10 days after surgery
Patients with neoplasm	71.8 ± 69.0*	53.3 ± 46.3^	63.2 ± 47.1	112.7 ± 93.8^
Patients without neoplasm	35.5 ± 32.1	20.1 ± 17.1	24.7 ± 25.5	32.6 ± 34.1
Healthy controls	23.1 ± 18.73			

Table 2. Serum sIL-2 levels in the individual groups before and after surgery

Study group	sIL-2R level before surgery U/ml	sIL-2R level three days after surgery U/ml	sIL-2R level seven days after surgery U/ml	sIL-2R 10 days after surgery U/ml
Patients with neoplasm	887.2 ± 665*	1046.7 ± 650^	931.7 ± 379^	893.7 ± 305^
Patients without neoplasm	909.3 ± 481.9	1189.7 754.5^	1159.6 ± 631.7^	1232.94 ± 683.5^
Healthy controls	717.4 ± 582			

Table 3. CRP and SAA levels in the individual groups before and after surgery

Study group	CRP and SAA levels before surgery	CRP and SAA levels three days after surgery	CRP and SAA levels seven days after surgery	CCRP and SAA levels 10 days after surgery
CRP Patients mg/dl	1.52 ± 1.89	9.19 ± 2.56^	5.55 ± 3.4^	5.38 ± 3.63^
CRP Controls mg/dl	0.53 ± 0.16			
SAA Patients µg/ml	82.54 ± 172.4*	961.6 ± 249.2^	545.1 ± 416^	502.8 ± 433^
SAA Controls µg/ml	3.17 ± 6.1			

* Significance in relation to control group, ^ Significance in relation to preoperative results

The results of acute phase CRP and SAA protein showed higher values in patients with bronchial carcinoma compared to healthy controls. The SAA protein values were found to be particularly high (82.54 in the patient group and 3.17 µg/ml in the controls) (Tab. 3). The postoperative values of these proteins rapidly increased just after surgery and gradually became normal in the successive examinations. However, after 10 days in both cases the values were higher than those in the control group. An extremely high increase in SAA protein after surgery is noteworthy (82.54 before and 961.6 µg/ml after the operation).

DISCUSSION

The surgical removal of tumors increases chances of complete destruction of "residual neoplastic disease" by cytotoxic lymphocytes, which enables to eliminate small numbers of neoplastic cells from the organism. However, the situation is not fully favorable since the surgical procedure itself, depending on its type and the underlying disease, induces immunosuppression. The operation results in impaired proliferative activity of lymphocytes, decreased IL-2 synthesis by T-lymphocytes, decreased expression of MHC class II antigens on monocytes, impaired phagocytosis and increased production of prostoglandine E_2 (9,19). The majority of the damaged tissues induce the release of acute phase proteins and are likely to modulate the release of relevant cytokines (6). The blood levels of IL-2 and CRP are proportional to the extent of tissue injury and are reversibly proportional to the degree of immunosuppresion. IL-6 and TNF regulate the CRP secretion, while IL-1 mainly the SAA proteins. The acute phase proteins together with IL-2 and IL-6 additionally activate cytotoxicity of NK and LAK cells and are involved in the activation of classical complement pathway (18). They act as opsonins for bacterial cells, fungi and parasites binding with high specificity the phosphorocholine particles on their surface or the surface of damaged endogenic cells (13). The increased levels of acute phase protein found in our study are consistent with the results observed in patients operated on for colorectal cancer (5), which may be interpreted as the immune response induced by injured tissues. The significance of decreased IL-2 levels in relation to the release of other cell mediators and the host response to injury has not been fully examined (explained). The role of IL-2 in antineoplastic resistance is connected with IL-2 stimulation of the release of other cytokines – IL-1, IL-4 and TNF-alpha, which was shown *in vitro* and *vivo* studies. IL-2 activates NK cells and cytotoxicity of LAK cells which are capable of inhibiting the development of metastases. A transitory decrease in IL-2 directly after surgery, which was observed in our study, is consistent with the results reported by other authors (4,7) and those showing an increase in the soluble receptor for IL-2 (5). This is favorable since the presence of the soluble form of receptors additionally decreases possible action of IL-2. For the purpose of immune prevention in the postoperative period IL-2 recombinant is administered (for up to 10 days) irrespective of the exogenous cytokine therapy (8) or cytokine genes are administered to the tumor cells to increase antineoplastic response (1).

In conclusion, our preliminary findings about immune indices after bronchial carcinoma surgery are found to be consistent with the results observed in patients subjected to surgery due to other neoplastic tumors. Decreased levels of serum IL-2 in the postoperative period need further studies concerning the postoperative administration of exogenous IL-2.

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SUMMARY

The aim of the study was to examine the levels of IL-2 and soluble receptor sIL-2R involved in the activation of TH1 lymphocytes, C-reactive protein (CRP) and serum amyloid A (SAA), whose synthesis is induced by IL-6 and IL-1, in the patients with bronchial carcinoma subjected to surgical procedures. The studies involved 35 patients operated on for bronchial carcinoma and 17 patients requiring thoracic surgery due to other reasons. The immune parameters were determined preoperatively and three, seven, 10 days after the operation. The control group consisted of 22 healthy individuals whose parameters were determined only once. The analysis revealed that the serum IL-2 level found in the patients was three times higher than that in controls. Moreover, the average sIL-2 level was also statistically higher in the group of patients. The results obtained three days after the procedure showed decreased IL-2 levels in both groups subjected to surgery (due to bronchial carcinoma or other reasons). The findings of subsequent determinations performed seven and 10 days after the operation showed that the values observed 10 days after surgery were higher than the preoperative ones. The sIL-2 levels three days after the operation significantly increased in both groups of patients, however, on next examinations they started to vary; the sIL-2R decreased in the bronchial carcinoma patients and increased in the other group. The results of CRP and SAA examinations revealed higher values in the bronchial carcinoma patients compared to controls. The SAA levels were

extremely high (82.54 in patients and 3.17μg/ml in controls). The postoperative levels of this protein showed a similar tendency – a rapid postoperative increase and gradual normalization in subsequent examinations. However, in both cases the values observed 10 days after the operation were higher than those in the control group. An extremely high increase in SAA levels after surgery (82.54-preoperatively versus 961.6 μg/ml–postoperatively) is noteworthy. Our preliminary results on the immune indices after bronchial carcinoma surgery are consistent with those observed after the operations for other neoplastic tumours; decreased levels of serum IL-2 in the postoperative period need further studies concerning the postoperative administration of exogenous IL-2.

Zachowanie się wskaźników aktywacji immunologicznej po operacji raka oskrzela

Celem pracy było zbadanie stężeń IL-2 i rozpuszczalnego receptora sIL-2R związanych z aktywacją subpopulacji limfocytów Th1 oraz białka C-reaktywnego (CRP) i białka amyloidowego (SAA), syntetyzowanych pod wpływem IL-6 i IL-1 u chorych operowanych z powodu raka oskrzela. Badania obejmowały 35 pacjentów operowanych z powodu raka oskrzela i 17 chorych wymagających leczenia operacyjnego w zakresie klatki piersiowej z innych przyczyn, stanowiących grupę kontrolną. Parametry immunologiczne były określane przed zabiegiem chirurgicznym oraz w trzecim, siódmym i dziesiątym dniu po operacji. Drugą grupę kontrolną stanowiły 22 zdrowe osoby, u których jednorazowo wykonano oznaczenie badanych parametrów. Analiza stężeń IL-2 w surowicy chorych i w grupach kontrolnych wykazała, że u chorych z rakiem oskrzela stężenie tej cytokiny jest trzykrotnie wyższe niż w grupie kontrolnej ludzi zdrowych. Średnie stężenie rozpuszczalnego receptora dla IL-2 w badaniach wykonanych w trzy dni po operacji wykazało spadek zarówno w grupie operowanych z powodu raka oskrzela, jak i operowanych z innych przyczyn. Wyniki kolejnych badań, wykonane w siedem i 10 dni, wykazały systematyczny wzrost stężenia IL-2, przy czym stężenie w grupie chorych z rakiem oskrzela po 10 dniach osiągnęło wartości wyższe niż przed operacją. Stężenia sIL-2R w trzy dni po operacji wzrosły w sposób istotny w obu grupach chorych, a zaczęły zachowywać się różnie w kolejnych badaniach; stężenia sIL-2R spadały u chorych z rakiem oskrzela, natomiast w drugiej nadal utrzymywała się tendencja wzrostu. Wyniki badań białek ostrej fazy CRP i SAA wykazały wyższe wartości u chorych z rakiem oskrzela w stosunku do wyników grupy kontrolnej ludzi zdrowych. Szczególnie wysokie wartości stwierdzono w stężeniach SAA (82,54 w grupie chorych w stosunku do 3,17 μg/ml w grupie kontrolnej). Zachowanie się tych białek po operacji wykazywało podobne tendencje – skokowy wzrost po operacji i tendencje stopniowej normalizacji wyników w kolejnych badaniach. W obu przypadkach po 10 dniach wartości były jednak wyższe niż w grupie kontrolnej. Zwraca uwagę szczególnie wysoki wzrost stężeń SAA po operacji (82,54 przed zabiegiem i 961,6 μg/ml po operacji). Nasze wstępne wyniki dotyczące zachowania się badanych wskaźników immunologicznych po operacji raka oskrzela są zgodne z wynikami w przypadku operacji innych guzów nowotworowych, a obniżenie IL-2 w surowicy w okresie pooperacyjnym sugeruje celowość badań nad podawaniem w tym okresie egzogennej interleukiny 2.