ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LX, N 1, 96 SECTIO D 2005

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Expression of lymphocytic Fas receptor and plasma soluble Fas in patients undergoing coronary artery bypass graft operation

There are many reports indicating that surgical trauma induces immune system dysfunction leading to profound but transient immunodepression. Depletion of all types of circulating lymphocytes may be observed in patients undergoing mayor operation, however the mechanism underlying this phenomenon is still unclear (3, 4, 7, 8, 10). Cells can dye in two ways, by apoptosis or necrosis, depending on type and intensity of noxious compounds. These two forms of cell death can be clearly morphologically distinguished. During necrosis, the cell swells, its mitochondria dilate, other organelles dissolve and the plasma membrane ruptures, releasing cytoplasmic material. This process often elicits an inflammatory response. By contrast, during apoptosis, the cytoplasm shrinks and the chromatin condenses, but the organelles retain their integrity (5, 6, 8, 9). Apoptosis is genetically programmed and strictly controlled cell death that is physiologically determined. It can be induced by many different stimuli, such as surgical stress and general anesthesia, especially during cardiovascular operations with extracorporeal circulation (ECC) where cells are, in addition to other changes in their functioning, mechanically damaged (2, 3, 4, 7, 8, 10).

In our study we investigated apoptosis of lymphocytes and its regulatory mechanisms such as the death-receptor pathway including Fas-Fas ligand complex. Fas (CD95) is a cell surface protein that belongs to the tumor necrosis factor (TNF) receptor superfamily. It is attached to the cell plasma membrane by a single transmembrane domain, while soluble form of Fas (sFas) lacks this domain. Fas receptor is expressed in high levels on various cells including T and B lymphocytes. Its ligand (FasL, CD95L) is a type II membrane protein homologous to TNF and belongs to the TNF superfamily. FasL can be found on activated T cells, especially on cytotoxic T cells and NK cells, which are able to induce apoptotic cell death on Fas-expressing lymphocytes. It is also reported that FasL sometimes mediates autocrine T cell suicide. This protein can be proteolytically cleaved from the cell membrane by a metalloprotease and occurs as a soluble forms – sFasL. Apoptosis is induced when FasL or sFasL binds to Fas receptor. It has been reported that a soluble form of Fas can block apoptosis by inhibiting this process (1, 8, 9).

In the immune system apoptosis of lymphocytes is one of important mechanisms regulating the extent and duration of immune response. This especially regards activated T lymphocytes and is described as activation-induced cell death (AICD). Lymphocytes that fulfil their physiological function must be eliminated from the circulation, in order to avoid autoimmunological disorders. AICD is assumed to play an essential role in the induction of peripheral tolerance and in downregulating of the immune response. This process is controlled by Fas system and executed by FasL. In this way Fas-Fas ligand complex acts as an important safeguard of the immune system that controls the expansion of activated T lymphocytes (1,11,12).

It is suggested that deleterious effects of mayor surgery especially coronary artery bypass graft (CABG) procedure are related to activation of lymphocytes and neutrophils, production of proinflammatory cytokines like IL-6, IL-8, TNF-α, free radicals generation, and many other reactions known as the systemic inflammatory response. Apoptosis and AICD process is responsible for the resolution of this inflammatory response but it contributes to the postsurgical immunosuppression.

In our study we measured changes in expression of Fas receptor on T lymphocytes and its soluble form sFas during and after CABG procedure.

MATERIAL AND METHODS

P a t i e n t s . The study subjects consisted of 12 patients aged from 53 to 72 years treated on stable angina pectoris and scheduled for nonurgent aortocoronary bypass (CABG-coronary artery bypass graft) with extracorporeal circulation (ECC). Patients were excluded from the study if they had received immunosuppressive drugs, such as corticosteroids, if they were known to have immunodeficiency syndromes, or received a blood transfusion within 12 hours preceding study enrollment.

M a t e r i a l s . Peripheral blood samples were taken seven times: 1) just before anesthesia, 2) 2 hours after the beginning of surgery, 3) immediately after surgery, 4) 6 hours after surgery, 5) 18 hours after surgery, 6) 30 hours after surgery, 7) 48 hours after surgery. The samples were immediately transferred to the laboratory for detection of apoptotic cells.

P r e p a r a t i o n o f c e l l s . Mononuclear cells were isolated by density gradient centrifugation (Gradisol, Aqua-Medica, Poland), washed twice and resuspended in PBS. The cells were then incubated for 30 min. with anti-CD3 FITC-conjugated monoclonal antibody and with anti-CD95 PerCP-conjugated monoclonal antibody (Caltag, USA). Excess, unbonded antibodies were removed by 2 washes with PBS and cells were immediately examined on a FACSCalibur flow cytometer (Becton Dickinson, USA) (Fig. 1).

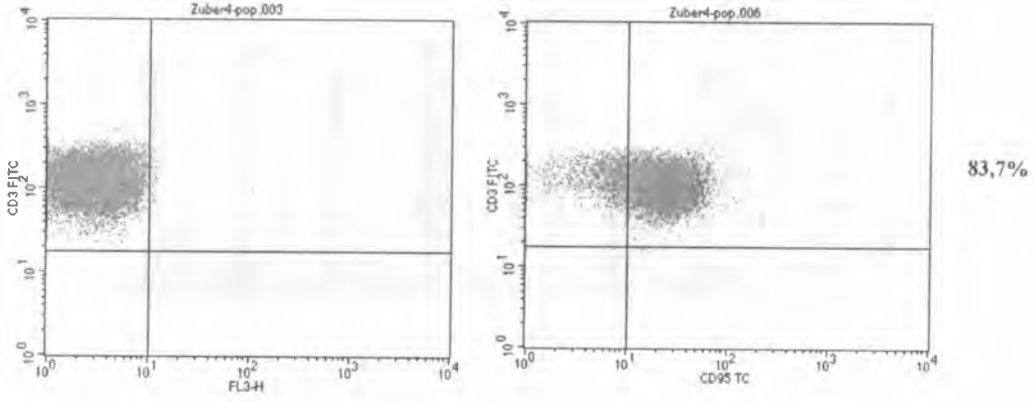

Fig.1. On the right: Flow cytometric analysis of CD95+ T lymphocytes (CD3+ cells). On the left: fluorescent dot plot from negative control sample- only CD3+ cells

Determination of sFas was carried out by means of enzyme-linked immunoabsorbent assay (ELISA) kit (Bender Med Systems). Peripheral blood serum samples were taken and stored at – 80°C till the time of examination. The detection limit of the test was 20 pg/ml.

Briefly, polystyrene plates were precoated with a rabbit immunoglobulin specific for sFas. Samples containing standard amounts of sFas as well as serum samples were added to individual wells. After three washes to remove unbound protein, a murine monoclonal antibody specific for sFas and conjugated to peroxidase was added to the wells. The wells were then washed once more time and substrate solution was added. The reaction was terminated by the addition of stop solution. The color generated was measured with spectrophotometry, calculated and presented as a protein plasma concentration (pg/ml).

Statistical analysis. The Wilcoxon paired test was applied to analyze differences in percentages of CD95+ T cells and sFas plasma concentration between stages of the experiment. The p-value <0.05 was considered significant. Data were presented as mean ± standard deviation and as mediana. The Statistica 6 software was used for all statistical procedures.

RESULTS

We observed significant changes in the number of CD95+T lymphocytes from the first to the following measurements. It got down in the second, third and increased again in the following checkpoints of measurement (Table 1, Fig. 2). sFas level in plasma changed in a little different way. It got down in the second, increased in the third, fourth and got down again in the fifth, sixth and seventh stages of experiment (Table 2, Fig. 3, 4).

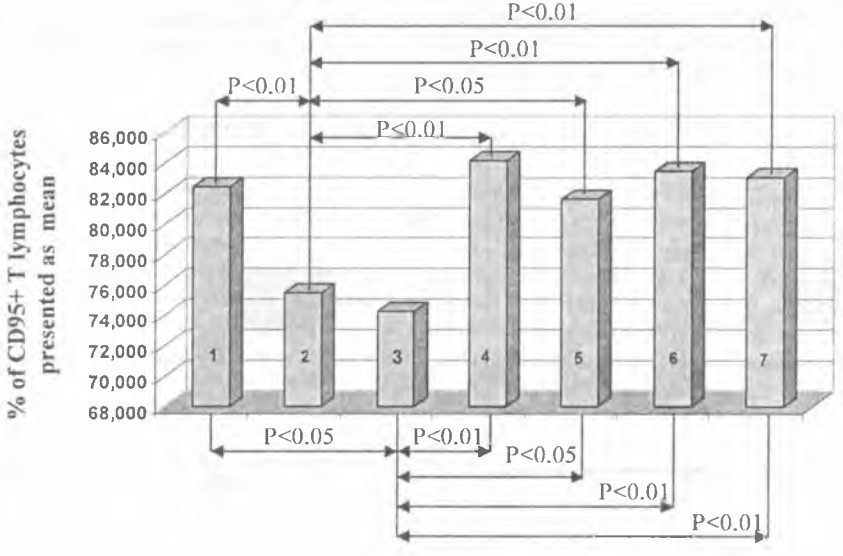

Fig. 2. Percentages of CD95+ T lymphocytes in subsequent measurements presented as mean ±standard deviation and as mediana

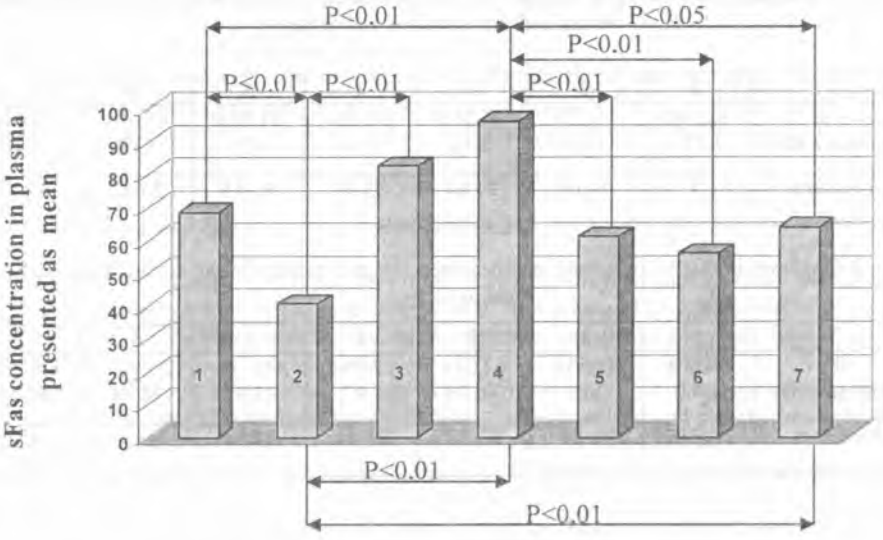

Fig. 3. sFas level in plasma in subsequent measurements presented as mean ± standard deviation and as mediana

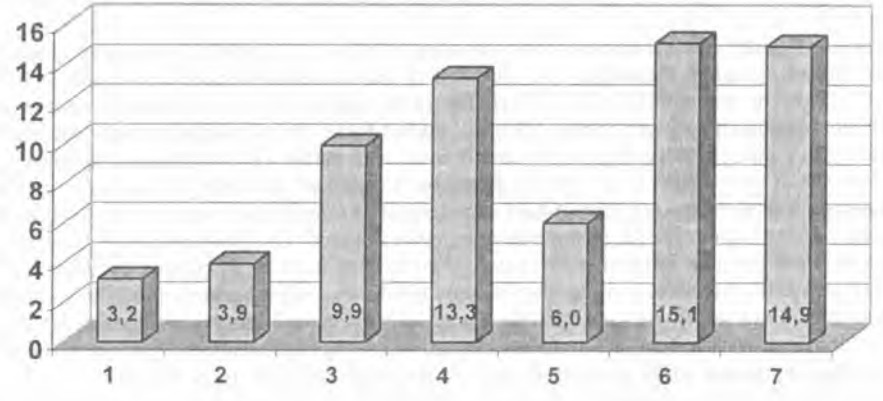

Fig. 4. Percentage of apoptotic cells measured by FDA in subsequent measurements presented as mean (from our previous study)

Tab. 1. Percentages of CD95+ T lymphocytes in subsequent measurements presented as mean ±standard deviation and as median

	1	2	3	4	5	6	7
Fas (mean±SD)	82,46± 3,71	75,52± 6,64	74,28± 7,36	84,12± 5,31	81,61± 4,25	83,48± 5,66	83,04± 4,84
Mediana	81,5	73,64	74,64	83,70	81,45	85,17	82,74

Tab. 2 sFas level in plasma in subsequent measurements presented as mean ± standard deviation and as median

	1	2	3	4	5	6	7
sFas (mean±SD)	68,46± 33,42	40,53± 20,1	82,37± 30,56	95,99± 41,9	60,93± 35,95	55,91± 37,51	63,79± 30,45
Mediana	54,74	36,89	83,39	85,44	54,94	40,13	58,92

DISCUSSION

The present study revealed that in patients undergoing CABG procedure expression of lymphocytic Fas receptor as well as its soluble form sFas changes in a characteristic manner from the first to the seventh stage of experiment. In our previous study performed on the same group of patients we also indicated dynamic changes in percentage of apoptotic lymphocytes during and after operation althought they were a little different especially as far as the second point of measurement (7).

Fas-Fas ligand system is considered an important mediator of apoptosis, especially apoptosis of activated T lymphocytes where it is referred to as activation-induced cell death (AICD) (1,11,12). There are many publications describing various aspects of immune system response and dysfunction during surgery or trauma. All these studies focus on immunodepression that occurs commonly in patients undergoing surgery and is associated partly with lymphocytic apoptosis or with necrosis of these cells (3, 4, 7, 8, 10). It has been suggested that lymphocytes from patients undergoing surgical trauma are susceptible to accelerated Fas-mediated apoptosis and that it might be a major factor responsible for postoperative lymphocytopenia. This phenomenon is more likely associated with systemic inflammatory response, significant increase of plasma cytokine levels like IL-6 and TNF-α, reactive oxygen intermediates (ROIs) and stress hormones including cortisol and catecholamines which are characteristic events of surgery and general anesthesia. All these factors contribute to activation of T-lymphocytes and following enhancement of plasma membrane Fas receptor expression which prepares these cells for apoptosis after cross-linking with FasL. In this way activated and potentially autoreactive T-cells are inhibited and eliminated from the circulation (3, 8).

In our study expression of Fas receptor on CD3+ T cells decreased in second and third checkpoints of measurement that may results from general dysfunction of immune system with disturbances in T-cell activation but more likely it results from excessive shedding of these receptors from the surface of cells leading to increase of sFas level in serum (in the third point of measurement). Shedding of the Fas receptor should be the most intensive in the second stage of experiment, during extracorporeal circulation (ECC) but ECC is associated with hemodilution and in consequence leads to decreased concentration of sFas receptor in plasma. In our previous study we noted onset of apoptotic lymphocytes in fourth stage that may result from excessive activation of T-cells after ending of the operation and following AICD reaction. Moreover, we observed previously significant decrese in number of apoptotic lymphocytes in the next fifth checkpoint of

measurement. Some explanation of this phenomenon may be the fact that sFas can block apoptosis by binding to FasL on effector cells and sFasL in plasma; so low level of sFas should in theory correlate with decreased percentage of apoptotic cells.

All these mechanisms involved in apoptosis of lymphocytes during CABG procedure have not been fully investigated yet. Further research is required for better understanding of this phenomenon because it is important for outcome of operation and patients recovery.

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SUMMARY

It is well known that surgical trauma induces immune system dysfunction leading to profound but transient immunodepression. This process resulted in cell necrosis or apoptosis. Apoptosis is genetically programmed and strictly controlled cell death that is physiologically determined. It can be induced by surgical stress, general anaesthesia, or some highly specialized procedures, such as extracorporeal circulation. The aim of this study was to analyze changes in expression of Fas (CD95) receptor on T lymphocytes and its soluble form sFas during and after CABG procedure. The study involved 12 men operated on due to stable coronary disease. The examinations were performed in seven stages: 1) just before anaesthesia, 2) 2 hours after the beginning of surgery, 3) immediately after surgery, 4) 6 hours after surgery, 5) 18 hours after surgery, 6) 30 hours after surgery and 7) 48 hours after surgery. The blood samples were immediately transferred to the laboratory for detection of apoptotic cells. The significant changes in the number of CD95+ T lymphocytes were noted from the first to the following measurements. It got down in the second and third and increased again in the following stages. sFas level in

plasma got down in the second, increased in the third, fourth and got down in the fifth to seventh stages. We conclude that coronary artery bypass graft operation resulted in T lymphocytes apoptosis. All these mechanism involved in apoptosis have have not been fully investigated yet.

Ekspresja receptora Fas na powierzchni limfocytów T i jego rozpuszczalnej formy sFas w osoczu pacjentów poddanych operacji pomostowania aortalno-wieńcowego

Istnieje wiele doniesień naukowych mówiących o występowaniu głębokich, chociaż przejściowych zaburzeń w obrębie układu immunologicznego w konsekwencji operacji prowadzonych w znieczuleniu ogólnym. Prace te skupiają się w większości na badaniu zjawiska pooperacyjnej limfocytopenii, która może być zależna od apoptozy bądź nekrozy tych komórek. Apoptoza jest genetycznie zaprogramowanym, ściśle kontrolowanym procesem, prowadzącym do naturalnej eliminacji komórek „zużytych", potencjalnie autoreaktywnych i mogących wywoływać zaburzenia autoimmunologiczne. Takimi komórkami są między innymi aktywowane limfocyty T. Po spełnieniu swojej biologicznej funkcji są one usuwane z krążenia na drodze apoptozy, zachodzącej w głównej mierze poprzez układ receptorowy Fas-FasL. W pracy mierzyliśmy zmiany ekspresji receptora Fas (CD95) na powierzchni limfocytów T oraz w osoczu 12 pacjentów leczonych z powodu stabilnej choroby wieńcowej i poddanych operacji pomostowania aortalno-wieńcowego (CABG) w znieczuleniu ogólnym z użyciem krążenia pozaustrojowego (ECC). Badanie przeprowadzono w siedmiu etapach: 1) przed wprowadzeniem do znieczulenia, 2) 2 godziny po rozpoczęciu operacji, 3) bezpośrednio po operacji, 4) 6 godzin po zakończeniu operacji, 5) 18 godzin po zakończeniu operacji, 6) 30 godzin po zakończeniu operacji, 7) 48 godzin po zakończeniu operacji. Zaobserwowaliśmy dynamiczne zmiany w odsetkach limfocytów T posiadających na swej powierzchni receptor Fas, jak również w stężeniach rozpuszczalnej formy receptora (sFas) w osoczu w kolejnych etapach badania. Wyniki naszych badań jak również doświadczeń przeprowadzonych uprzednio potwierdzają hipotezę, zgodnie z którą pooperacyjne zaburzenia funkcji układu immunologicznego, spadek odporności i podatność pacjentów na infekcje spowodowane są apoptozą komórek immunokompetentnych, w tym limfocytów T. W zjawisko to zaangażowany jest bezpośrednio układ receptorowy Fas-FasL.