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Enhanced spontaneous antibody response after coronary artery bypass surgery

Purpose: Cells spontaneously secreting immunoglobulins can be seen in the blood one week after open-heart surgery. The purpose of this study was to measure the antibody specificities of activated cells.

Methods: Immune responses were studied preoperatively and on the seventh postoperative day in 18 patients undergoing elective coronary artery bypass surgery. The number of cells secreting adenovirus, measles, rubella and tetanus antigen specific antibodies spontaneously and induced by pokeweed mitogen PWM (ASCs) as well as the total number of cells secreting IgG, IgM and IgA (ISCs) were studied using an enzyme-linked immunospot (ELISPOT) assay. Spontaneous as well as phytohaemagglutinin (PHA)- and pokeweed mitogen (PWM)-induced lymphocyte proliferation was also measured.

Results: The number of cells spontaneously secreting IgG, IgM and IgA antibodies was increased on the seventh day after coronary bypass surgery, against adenovirus, measles, rubella and tetanus as well as the total number of cells secreting immunoglobulins IgG, IgM and IgA ($P < 0.05/0.001$). By contrast, only slight fluctuation was seen in the numbers of cells secreting antibodies after PWM stimulation. Spontaneous lymphocyte proliferation was also increased, PHA proliferative responses were depressed and PWM responses were not changed on the seventh postoperative day compared with preoperative values.

Conclusion: Coronary artery bypass surgery caused marked polyclonal B cell activation demonstrated by an increase of cells producing spontaneously antibodies against virus antigens and tetanus toxoid. This activation could not be intensified by PWM stimulation.

Objectif : Une semaine après une chirurgie à coeur ouvert, on retrouve dans le sang des cellules qui sécrètent des immunoglobulines. Cette étude visait à mesurer la spécificité des anticorps propres aux cellules activées.

Méthodes : Les réponses immunitaires de 18 patients ont été étudiées avant une chirurgie de revascularisation myocardique non urgente et sept jours plus tard. Le nombre de cellules sécrétant spontanément des anticorps spécifiques aux adénovirus, à la rougeole, à la rubéole et au tétanos et les anticorps induits par le mitogène pokeweed PWM (ASC) ainsi que le nombre total des cellules sécrétant des IgG, IgM et IgA (ISC) ont été étudiées avec une épreuve d'immunospot lié aux enzymes (ELISPOT). En plus de la prolifération spontanée, la prolifération lymphocytaire induite par la phytohémagglutinine (PHA) et par le mitogène pokeweed a été mesurée.

Résultats : Le septième jour après l'intervention, on constatait une augmentation du nombre de cellules sécrétant spontanément des anticorps IgG, IgM et IgA contre les adénovirus, la rougeole, la rubéole et le tétanos aussi bien que du nombre de cellules sécrétant des immunoglobulines IgG, IgM et IgA ($P < 0.05/0.001$). Par contre, seulement de légères fluctuations ont été notées dans le nombre de cellules sécrétant des anticorps après la stimulation par le PWM. La prolifération lymphocytaire spontanée augmentait aussi, les réponses PHA prolifératrices étaient déprimées et les réponses PWM ne changeaient pas la septième journée postopératoire comparativement aux valeurs préopératoires.

Conclusion : La chirurgie de revascularisation myocardique provoque une importante activation polyclonale des cellules B qui se manifeste par une augmentation des cellules produisant spontanément des anticorps contre les antigènes viraux et la toxoïde tétanique. Cette activation n'est pas intensifiée par la stimulation par le PWM.

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IMMUNOLOGICAL homeostasis is affected by anaesthesia and surgery. The specific immune response shows lymphopaenia, depressed cell-mediated immunity and alterations in B cell responses.¹ Spontaneous proliferation of lymphocytes increases over the first seven days after open-heart surgery^{2,3} and other types of surgery.⁴ Increased numbers of cells spontaneously secreting IgG, IgM and IgA have been simultaneously observed on the seventh postoperative day in the peripheral circulation.³ This study was carried out to characterize this activation by measuring the antibody specificities of these surgery-activated cells. The number of cells secreting specific IgG, IgM and IgA antibodies spontaneously and after pokeweed mitogen (PWM) induction was calculated. A panel of ubiquitous antigens was chosen which contained measles, rubella and adenovirus, and tetanus toxoid against which an extensive immunization program in the Finnish population has been conducted.

Methods

Patients

The study was conducted according to the principles on human experimentation of the Helsinki Declaration and approved by the Ethical Committee of the Turku University Medical Faculty and the Turku University Central Hospital. Informed consent was obtained from each patient. Eighteen male patients (mean age 60 ± 10 (SD) yr, weight 88 ± 12 kg, height 178 ± 8 cm and ASA class 4) undergoing elective coronary artery bypass surgery were studied. Concomitant medication including long-acting nitrates, calcium channel blockers and β -adrenergic receptor blockers was continued until the morning of surgery. Patients with a history of malignant, haematological or endocrinological disease or who were receiving immunosuppressive drugs were excluded.

The operations were carried out under moderate-dose fentanyl anaesthesia supplemented with lorazepam, isoflurane and pancuronium. The patients received $6 \mu\text{g}\cdot\text{kg}^{-1}$ scopolamine and $0.2 \text{ mg}\cdot\text{kg}^{-1}$ morphine sc as preanaesthetic medication 60–90 min before operation and at induction of anaesthesia 4 mg lorazepam and $30 \mu\text{g}\cdot\text{kg}^{-1}$ fentanyl, followed by an infusion of $0.1\text{--}0.2 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$ fentanyl. The inspiratory concentration of isoflurane was $0.2\text{--}1.5$ v/v %. Mechanical ventilation was used with oxygen 40% in air. Moderate (30°C) systemic hypothermia was used during extracorporeal circulation. The roller-pump heart-lung-machine with a hollow fibre oxygenator (Dideco D 703 Compactflo System; Dideco S.p.A., Mirandola, Italy), cardiectomy reservoir/venous reservoir and customized polyvinyl chloride tubing including an arterial line leucocyte nondepleting filter (Micro

20A, Dideco) was primed with 2000 ml of a crystalloid solution and 100 ml of mannitol 15%. The flow rate was $2.4 \text{ l}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$ body surface area, reduced to $1.9 \text{ l}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$ during hypothermia. Anticoagulation was maintained using porcine heparin (initial dose $3 \text{ mg}\cdot\text{kg}^{-1}$). Activated clotting time was maintained over 400 sec throughout the extracorporeal circulation. Cardioplegic arrest was achieved with cold (4°C) St. Thomas's solution (1000 ml) and local cooling with ice-slush in the pericardial cradle. Invasive central haemodynamics were monitored during and after operation using a pulmonary artery catheter and a radial artery cannula. In addition, the ECG, oxygen saturation and end-tidal CO_2 concentrations were monitored. No remarkable haemodynamic instability occurred in any of the patients. No corticosteroids were used. The median number of grafts was three (range 2 – 5), and the mean duration of anaesthesia, surgery and extracorporeal perfusion was 234 ± 31 (SD) min, 176 ± 30 min and 99 ± 28 min, respectively. Autologous blood transfusion was used, and all except one patient also received Sagman packed red blood cells, median two units (range one – nine). After operation, the lungs were ventilated in normocapnia until the first postoperative morning and then the tracheas were extubated. The patients received 1.5 g cefuroxime *iv* before operation and 750 mg three times daily for three postoperative days. No operative or other complications or infections occurred.

In addition, five healthy volunteers from the hospital staff (mean age 40 ± 9 (SD) yr, weight 78 ± 14 kg and height 179 ± 8 cm) were used as a control group to test the stability of the methods. The control group volunteers did not differ in their background data from the patients (Student's *t* test).

Blood samples

Processing of the heparinized venous blood samples, taken on the morning before operation and on the morning of the seventh postoperative day, was started immediately after collection. Haemoglobin concentrations, haematocrit as well as leucocyte and differential counts were determined using the H6000 blood cell analyzer (Technicon Instruments Co., Tarrytown, NY, USA). Mononuclear cells were separated in Ficoll-Isopaque (Ficoll-Paque,[®] Pharmacia Fine Chemicals, Uppsala, Sweden) by centrifugation at 400 g for 40 min at $+4^\circ\text{C}$ and then washed twice with Hanks' balanced salt solution and diluted in RPMI 1640 medium (Gibco, Grand Island, NY, USA).⁵ The purity of the mononuclear cell yield was evaluated, and viability of cells over 95% was determined by the trypan blue exclusion method.

Measurement of antibody secreting cells

The number of cells showing spontaneous and PWM-inducible specific antibody secretion cells (ASCs) and the number of immunoglobulin secreting cells (ISCs) in blood samples were measured using an enzyme-linked solid-phase immunospot (ELISPOT) assay.⁶ The method has been described recently in the measurement of virus-specific ASCs.⁷

Briefly, for the calculation of specific ASC numbers, flat-bottomed 96-well microtitre plates (Nunc-Immuno Plate Maxisorp,TM Nunc A/S, Roskilde, Denmark) were precoated with 50 µl of the antigens including adenovirus hexon 5 µg·ml⁻¹(⁸), measles virus lysate 40 µg·ml⁻¹(⁹), rubella semipurified virions 20 µg·ml⁻¹(¹⁰) or tetanus toxoid 5 µg·ml⁻¹ (National Public Health Institute, Helsinki, Finland). For the measurement of ISC numbers, the plates were coated with 50 µl goat antihuman IgG (Zymed, San Francisco, Ca, USA), IgM (Dako, Glostrup, Denmark) or IgA (Dako) by incubating them for two hours at 37°C). After washing and blocking of nonspecific binding with bovine serum albumin (BSA)-PBS (phosphate-buffered saline), the plates were incubated with 50 µl of three dilutions of isolated mononuclear cell suspension (2×10^5 , 1×10^5 or 0.5×10^5 cells·well⁻¹ for determination of antigen specific ASC numbers and 1×10^4 , 0.25×10^4 or 0.062×10^4 cells·well⁻¹ for determination of ISC numbers in quad replicate). After two hours of incubation at 37°C, the plates were washed three times with PBS-Tween, and 50 µl alkaline phosphatase-conjugated antihuman IgG (Orion, Espoo, Finland for ASCs and Sigma, St Louis, MO, USA for ISCs), IgM or IgA (Orion) diluted 1:100 in PBS+1% BSA was added for determination of specific ASC and ISC numbers. The plates were then incubated overnight at 4°C. After another four washes with PBS-Tween, 50 µl substrate-agarose were overlaid.

Substrate-agarose was prepared as follows: 0.6 g low-electroendosmosis agarose (Sigma) was boiled in 20 ml distilled water until completely dissolved. Then, 5 ml 5-bromo-4-chloro-3-indolylphosphate (Sigma) dissolved in 2-amino-2-methyl-1-propanol (Fluka, Buchs, Switzerland) was added to the above 20 ml of agar 3% solution, after which substrate-agarose was ready for use. The ASCs and ISCs were observed after overnight incubation in the dark at room temperature and visualized as dark blue spots under a stereomicroscope. The results are given as ASC or ISC numbers per 10^6 isolated mononuclear cells.

For the assessment of antibody secreting cells after PWM stimulation 2×10^6 separated mononuclear cells were first cultured in 2 ml test tubes with 10 µg·ml⁻¹ PWM in humidified air and CO₂ 5% at 37°C for seven

days. Then, the same assay as that for measurement of cells spontaneously secreting antibodies was used.

Measurement of lymphocyte proliferative responses

Separated mononuclear cells (1×10^5) were cultured in quadruplicate in RPMI 1640 (Gibco) with 50 µg·ml⁻¹ gentamicin, AB serum 10% and mitogens in wells of flat-bottomed microtitre plates in humidified air and CO₂ 5% at 37°C for seven days.¹¹ The culture mitogens were 100 µg·ml⁻¹ phytohemagglutinin (PHA, Difco Detroit, MI, USA) and 2.5 µg·ml⁻¹ pokeweed mitogen (PWM, Gibco). The volume of each culture was 200 µl. Eighteen hours before harvesting, 1 µCi of ³H-thymidine (specific activity 98 mCi·mg⁻¹, Radiochemical Centre, Amersham, UK) in 20 µl was added to each microculture. Radioactivity was measured as disintegrations per minute (dpm) of ³H-thymidine incorporation into DNA, using a scintillation counter (LKB-Wallac, Turku, Finland).

Statistics

The Wilcoxon matched paired test was used to compare pre- and postoperative values. Student's *t* test and the Mann-Whitney U test were used to compare preoperative values between patients and volunteers who served as methodological controls.

Results

The number of leucocytes, percentage of neutrophils, eosinophils and basophils were increased and the percentage of lymphocytes decreased on the seventh postoperative day from preoperative values ($P < 0.05$ – 0.001) (Table I). The absolute numbers of lymphocytes in the circulation were at the preoperative level on the

TABLE I Blood haemoglobin concentrations, haematocrit and blood cell counts in patients before operation (I) and on the seventh postoperative day (II). Means (SD), *n*=18.

	I		II		
Haemoglobin, g · l ⁻¹	141	(10.4)	127	(11.3)	***
Haematocrit	0.41	(0.03)	0.38	(0.03)	**
Erythrocytes, $\times 10^{12} \cdot l^{-1}$	4.42	(0.38)	4.08	(0.41)	***
Leucocytes, $\times 10^9 \cdot l^{-1}$	6.4	(1.7)	8.1	(2.4)	***
Neutrophils, %	59.6	(7.3)	63.9	(6.4)	*
Neutrophils, $\times 10^9 \cdot l^{-1}$	3.84	(1.26)	5.19	(1.70)	***
Lymphocytes, %	28.5	(5.9)	21.9	(6.9)	***
Lymphocytes, $\times 10^9 \cdot l^{-1}$	1.77	(0.46)	1.77	(0.67)	
Monocytes, %	7.4	(1.1)	7.4	(1.8)	
Monocytes, $\times 10^9 \cdot l^{-1}$	0.48	(0.16)	0.60	(0.23)	*
Eosinophils, %	2.1	(1.2)	3.4	(1.6)	***
Eosinophils, $\times 10^9 \cdot l^{-1}$	0.14	(0.08)	0.28	(0.14)	***
Basophils, %	0.8	(0.4)	1.1	(0.2)	*
Basophils, $\times 10^9 \cdot l^{-1}$	0.06	(0.03)	0.08	(0.03)	***

Differences between pre- and postoperative values (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

seventh postoperative day. A postoperative decrease was also observed in haemoglobin concentration, haematocrit and erythrocyte count ($P < 0.05-0.000$).

The number of cells secreting spontaneously IgG, IgM and IgA against adenovirus, measles, rubella and tetanus (ASC) as well as the total numbers of cells spontaneously secreting IgG, IgM and IgA (ISC) had increased by the seventh postoperative day after coronary artery bypass surgery from preoperative values ($P < 0.05-0.001$) (Figure 1). By contrast, similar changes were not seen after PWM stimulation in ASC or ISC numbers. The tests showed decreased numbers of PWM-stimulated IgG and IgM ASCs against adenovirus ($P < 0.05$) and increased numbers of IgA ASC against rubella on the seventh postoperative day ($P < 0.05$). No alterations were seen in the numbers of ASCs against measles or tetanus or in any of the ISC numbers.

Spontaneous lymphocyte proliferation, characterized by ^3H -thymidine uptake, was increased on the seventh postoperative day ($P < 0.05$), but PHA-induced lymphocyte proliferative responses were depressed compared with preoperative values ($P < 0.05$). No differences were seen in PWM-induced responses between pre- and postoperative values (Table II).

No differences were seen between the patients and controls in any of the test values preoperatively, and no alterations were observed in any of the test values of the controls from the first to the second blood sample.

Discussion

This study measured antibody specificities of surgery-activated cells, not the capacity of the body to respond to microbial or other antigens during or after operation. The focus was on the increased number of immunoglobulin secreting cells found in the circulation about one week after the operation, disregarding any immediate postoperative alterations in the immune response. The study confirmed the earlier finding of an increased number of cells spontaneously secreting IgG, IgM and IgA one week after open heart surgery.³ A novel finding was the several fold increase in the number of IgG, IgM and IgA specific antibody secreting cells (ASC) against all antigens of different types (adenovirus, measles, rubella, and tetanus). This activation was more intensive than that induced by PWM. The activation affected all specific ASCs alike, supporting the hypothesis of nonspecific spontaneous polyclonal B cell activation after open-heart surgery. In accordance with our results, DiPadova *et al.* found production of antibodies against enterococcal and pneumococcal antigens in mononuclear cell cultures seven days after major surgery.¹²

FIGURE 1 Numbers of cells showing spontaneous and PWM-induced secretion of IgG, IgM and IgA anti-adenovirus, -measles, -rubella, and -tetanus and total numbers of immunoglobulin secreting cells before operation (I) and on the seventh postoperative day (II). Geometric means, $n=18$.

Differences between pre- and postoperative values (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

TABLE II Lymphocyte proliferative responses as disintegrations per minute (dpm) before operation (I) and on the seventh postoperative day (II). Median (25th and 75th percentile), $n=18$.

	<i>I</i>		<i>II</i>	
Lymphocyte proliferative responses, dpm				
Unstimulated	282	(199–341)	460	(341–833)*
PHA-induced	159108	(98835–371197)	110301	(55032–215520)*
PWM-induced	122691	(56521–239043)	117854	(39193–190213)

Differences between pre- and postoperative values (* $P < 0.05$).

Polyclonal B-cell activation has been seen during a vigorous immune response and during several viral and bacterial infections.¹³ Preferential synthesis of some viral antibodies, e.g., those specific for measles and rubella viruses, has been described.¹⁴ In this study the most prominent increase was seen in rubella virus antibodies. The response can be considered part of the nonspecific systemic inflammatory response associated with cardiac surgery and extracorporeal circulation¹⁵ and it includes activation of the complement, coagulation, fibrinolytic and kallikrein cascades, activation of neutrophils, free oxygen radical release and cytokine response. Among these factors, several cytokines, including $\text{IL-1}\alpha$, $\text{IL-1}\beta$ and $\text{TNF}\alpha$, stimulate B-cell responses and IgG, IgM and IgA secretion.¹⁶ Plasma cytokine concentrations were not measured, but pro-inflammatory (IL-1 , IL-8 and $\text{TNF}\alpha$) and anti-inflammatory cytokines (IL-10) have been found to be

increased by open-heart surgery.¹⁷ An association may occur between the spontaneous polyclonal B cell activation shown in this study and the cytokine response generated by surgical trauma, extracorporeal circulation and leakage of endotoxin into the blood circulation during cardiopulmonary bypass surgery.¹⁸ Surgery alone, however, may be sufficient to produce the changes, as increased secretion of IgG and IgA has been seen seven days after cholecystectomy.¹² After surgery, decreased production of T helper cell type 1 (TH1) cytokines IL-2 and IFN γ ¹⁹ and increased production of TH2 cytokines IL-4²⁰ and IL-10¹⁷ has been described. This shift towards TH2 cells favours humoral immunity²¹ and may be the underlying mechanism of B-cell activation. Some drugs, including high dose fentanyl¹⁷ heparin²² and possibly β -adrenergic blocking drugs and calcium channel antagonists,²³ used by these patients may also have an indirect effect by altering cytokine responses.

The finding in this study of lymphocytes with increased spontaneous proliferative activity characterized by increased ³H-thymidine uptake seven days after operation is in agreement with the other findings of this study. These DNA-synthesizing cells are possibly the same cells as the spontaneous ASCs and ISCs measured in this study. The increase in spontaneous proliferative activity can already be observed after coronary artery bypass surgery on the first postoperative day, and the response is over by the 10–14th postoperative day.²

The increased numbers of cells spontaneously secreting specific antibodies (ASCs) and immunoglobulin secreting cells (ISCs) can be expected to increase antiviral and antibacterial resistance and to maintain immunity against antigens to which the body has earlier been exposed. It can thus be proposed that surgery, as a stress situation, activates memory cells, and found in this study as polyclonal B-cell activation, to maintain the immune status in humans. Polyclonal B-cell activation may also be involved in the pathogenesis of autoimmune disease.²⁴ On the other hand, the postoperative depression of cell-mediated immunity observed in this study as depressed PHA-induced lymphocyte proliferative responses is thought to protect the body from reacting against antigenic structures exposed during operation.² It is thus possible that disturbed balance between polyclonal B-cell activation and depressed cell-mediated immunity after surgery may arouse autoimmune responses in patients prone to autoimmune disease. Although autoimmune disease occurs rarely, this should, however, be investigated in epidemiological studies.

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