Eur J Cardio-thorac Surg (1996) 10: 61–67

ORIGINAL ARTICLE

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An imbalance in T-helper cell subsets alters immune response after cardiac surgery

Received: 27 September 1994
Accepted. 27 February 1995

Presented at the 8th Annual Meeting of the European Association for Cardio-thoracic Surgery, The Hague, The Netherlands, September 25–28, 1994

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Abstract Growing evidence indicates that cell-mediated immunity is altered after cardiac surgery with cardiopulmonary bypass (CPB). The objective of this prospective randomized study was to investigate (1) if an imbalance in T-helper cell (TH) subsets, i.e. TH1/TH2, may be responsible for these alterations and (2) if they can be counteracted. Twenty patients formed control group A. Twenty group B patients received indomethacin and thymopentin for immunomodulation. In vitro tests included measurements of TH, interleukin (IL)-2 as a cytokine primarily produced by TH1 cells, and IL-6 as a cytokine primarily produced by TH2. Delayed-type hypersensitivity (DTH) skin response and specific antibody (AB) production were used as in vivo tests for TH1- and TH2-induced immune response, respectively. Postoperatively, group A patients showed a persistent, significant reduction of TH, IL-2 synthesis and DTH skin response as compared to baseline values, while IL-6 synthesis remained unaltered and AB production increased ($P<0.05$). In group B patients no change in TH, IL-2 and IL-6 synthesis, or DTH skin response was observed ($P<0.05$ vs A). Postoperative AB production increased significantly in group B. These results indicate a significant suppression of TH1-induced cell-mediated immune response following CPB, while TH2-induced response remains normal. A normal TH2 response may be helpful for recovery following cardiac surgery by cleaning the body of the by-products of CPB. A suppression of TH1 response may gain clinical significance whenever a postoperative infection requires this response, but can be effectively counteracted by immunomodulatory intervention with indomethacin and thymopentin. [Eur J Cardio-thorac Surg (1996) 10: 61–67]

Key words Cardiopulmonary bypass · Cell-mediated immunity · TH1/TH2 imbalance · Immunomodulation

Introduction

During the last decade, growing evidence has suggested that cardiopulmonary bypass (CPB) induces substantial alterations within cell-mediated immunity (CMI) in virtually every patient undergoing cardiac surgery [1, 3, 6, 8, 15]. The clinical implications of these findings are apparently limited, since the majority of patients recover without any lasting sequelae from cardiac operations. One possible explanation for these contradictory findings could be that the observed alterations within CMI regulation indicate a physiologic response of the CMI system to the immunologic challenge represented by the extracorporeal circulation. During CPB the CMI system may become involved in the clearance of cell debris, denaturated proteins and other by-products acting as extracellular antigens. The adequate CMI response to these antigens is initiated by a

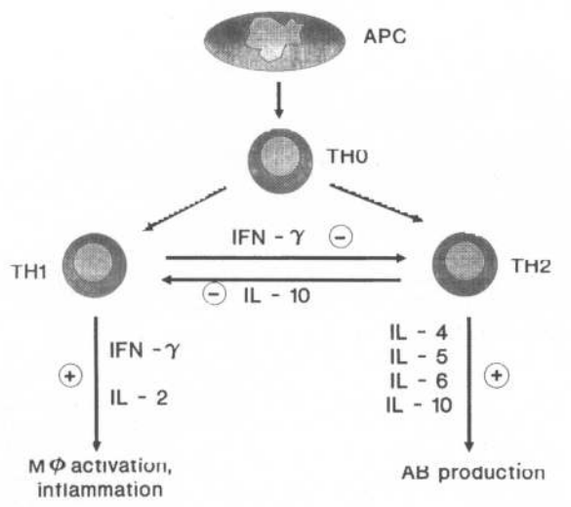


Fig. 1 Simplified model of the activation sequence of cell-mediated immunity. The antigen presenting cell (APC) activates the resting T-helper cell (TH0) to differentiate predominantly into a type 1 helper T cell (TH1) or a type 2 helper T cell (TH2). Subsequently TH1 mediates an inflammatory CMI response by synthesis and release of interleukin-2 (IL-2) and interferon-gamma (IFN-gamma). In addition, TH2 is inhibited by IFN-gamma. Type 2 helper T cell-mediated response leads to specific antibody (AB) production by synthesis and release of IL-4, -5, -6, and IL-10. Interleukin-10 additionally inhibits TH1 response

T-helper cell subset called type 2 helper T cell or TH2, which provides B cell help [13, 14, 16–18, 20]. The other subset, called type 1 helper T cell or TH1, is primarily activated by intracellular antigens such as bacteria or viruses.

One particularity of this T-helper cell dichotomy is the fact that one type of CMI response inhibits the alternate response to a certain degree, which is illustrated in Fig. 1. As shown in this simplified model, the antigen presenting cell (APC) activates the resting T cell (TH0). Presumably depending on the type of antigen presented and/or the APC and its cytokines, either the TH1 or the TH2 pathway of CMI response is initiated. The two pathways result in different cytokine synthesis patterns, and different mechanisms of antigen elimination, which is either an inflammatory response (TH1) primarily mediated by macrophages (M0), or a humoral response (TH2) mediated by specific antibodies (AB). In previous studies published on this subject by our group [8, 9] we focused only on forward regulatory and downregulatory pathways of TH1 response. The influence of the TH2 response and its interaction with TH1 reactions in patients having undergone cardiac surgery have not yet been addressed in the literature, either by our or another group.

The aim of this study, therefore, was to investigate (1) if the alterations within CMI represent an imbalance in T-helper cell subsets and (2) if these alterations can be counteracted by immunomodulatory intervention. In vitro, the percentage of CD4+ T-helper cells as well as the cytokines interleukin (IL)-2, which is primarily produced by TH1, and IL-6, which is primarily produced by TH2, were measured. In vivo, the delayed-type hypersensitivity (DTH) skin response and specific antibody (AB) production following tetanus vaccination were used to assess TH1 response (DTH) and TH2 response (AB production), respectively. For immunomodulation, indomethacin (Confortid)[1] was used to inhibit synthesis of prostaglandin E2 which was found to adversly affect T cell function [5, 11]. In addition, the synthetic thymus hormone thymopentin (Timmunox)[2] was given to enhance T cell activity [22].

Patients and methods

Forty patients undergoing either coronary artery bypass grafting ($n=31$) or heart valve replacement ($n=9$), older than 55 years and without evidence of a concomitant malignant or immunologic disease, were studied. The study had been approved by the Ethics Committee of the Faculty of Medicine at the Ludwig-Maximilians-University, Munich, Germany, and each patient had given informed consent. The mean age of the patients was 63 3±6.6 years, 11 patients were female and 29 male The mean left ventricular ejection fraction was 61±15%. The majority of patients suffered from coronary heart disease ($n=31$), while the remaining nine patients had valvular diseases: affecting the aortic valve in eight patients and the mitral valve in one.

Anesthetic drugs used were etomidate, fentanyl, pancuronium, and nitrous oxide to which isoflurane was added, if necessary. For antibiotic prophylaxis either ceftriaxone ($n=30$) or ciprofloxacin ($n=10$) was used. Cardiopulmonary bypass equipment consisted of roller pumps, disposable Bentley Bio-10 ($n=5$) or 10 plus ($n=1$) bubble oxygenators[3] or Sarns SMO ($n=18$)[4] and Medtronic Maxima ($n=16$) membrane oxygenators[5]. The pump was primed with 2 l standard electrolyte solution, 50 mmol sodium bicarbonate, 25 g Mannitol, 5000 IU heparin and 2 million U aprotinin. Moderate hypothermia (28 °C) was employed in all cases and pump flow rates were maintained at between 2 2 and 2.4 l/min per m^2. After the aorta had been cross-clamped, cold crystalloid cardioplegic solution was administered. We used either 700 ml Kirklin solution ($n=29$) or 2000 ml Bretschneider solution ($n=11$). The mean duration of aortic cross-clamping was 35.7±13 9 min. Total CPB time was 67 0± 26.7 min.

The mean number of distal coronary artery anastomoses was 2.35±0 64 For valve replacement we used four mechanical valves (St. Jude medical bileaflet[6], $n=3$, and Björk-Shiley Monostrut[7], $n=1$), and 5 porcine bioprosthesis (Hancock[8], $n=2$ and Carpentier-Edwards Supraannular[9], $n=3$). For the prospective randomized trial, the patients were divided into two subgroups: 20 group-A patients underwent conventional postoperative therapy, while 20 group-B patients received 100 mg indomethacin intravenously 6 h after the operation and 50 mg three times daily until postoperative day 5. In addition to indomethacin therapy, 50 mg thymopentin was given sub-

[1] Dumex, Copenhagen, Denmark
[2] Cilag, Sulzbach, Germany
[3] Baxter Healthcare Corp., Bentley Division, Irvine, Calif., USA
[4] Sarns Inc /3 M, Ann Arbor, Mich., USA
[5] Medtronic Blood Systems Inc., Anaheim, Calif , USA
[6] St. Jude Medical Inc., St. Paul, Minn., USA
[7] Shiley Inc., Irvine, Calif., USA
[8] Medtronic Cardiopulmonary Division, Anaheim, Calif., USA
[9] Baxter Healthcare Corp , Edwards Division, Santa Ana, Calif , USA

cutaneously 2 h before the operation, as well as on postoperative days 2 and 4. There were no significant differences in preoperative or operative data among the two groups. In particular, patients who received different antibiotics, underwent operation using two different types of oxygenators or cardioplegic solutions, and the implanted porcine or mechanical valves were randomly distributed.

Immunologic investigations

Preparation of peripheral blood mononuclear cells (PBMC). Blood samples were taken 2 days before the operation as well as on postoperative day 1 and 7. For lymphocyte studies, 60 ml of peripheral blood was obtained in sterile heparinized tubes from the patients. The blood samples were diluted in 1:2 Hanks buffered saline solution[10] with 2% penicillin/streptomycin added. Peripheral blood mononuclear cell isolation was performed immediately by standard Ficoll-Hypaque density gradient centrifugation at 1500 rpm, 4 °C for 25 min After the cells had been resuspended with 15% fetal calf serum, cell counts were performed with the hemocytometer with 0.1% trypan blue exclusion as a test of viability. Viability always exceeded 95%.

Peripheral blood mononuclear cells phenotyping. Phenotyping was performed with the monoclonal antibody OKT4[11] to quantify CD4+ helper/inducer T cells. The number of cells that were stained with the antibody was assayed by fluorescence microscopy.

Interleukin-2 and IL-6 assay. Interleukin-2 and IL-6 were generated by culturing PBMC suspensions at a cell concentration of 4×10^6 PBMC/ml in the presence of highly purified phytohemagglutinin (PHA) at a final concentration of 2.5 µg/ml. After 48 h, supernatants were collected and stored at –80 °C until assayed. The assay for the detection of IL-2 activity was a modification of the method described by Gillis and associates [4]. This bio-assay measures the proliferation of IL-2 dependent target cells. As target cells, PBMC of a healthy volunteer were used, which had been stimulated with concanavalin A. Proliferation of the target PBMC was assessed by measuring ^{3}H-thymidine incorporation, and compared to the proliferation induced by a reference-serum with known IL-2 activity. Interleukin-6 was measured employing the MTT assay [12] as decribed previously [7] using a murine hybridoma cell line (7TD1) that grows only in the presence of IL-6. This bio-assay measures the MTT-formazan production of the 7 TD1 cells as a parameter of IL-6-dependent cell proliferation. MTT-formazan production was assessed colorimetrically at a wave length of 550 nm. The proliferation induced by recombinant human IL-6 was used as reference.

Delayed-type hypersensitivity skin response. The DTH skin reactions to seven recall antigens were tested 2 days before the operation and on postoperative day 7. An antigen skin test battery[12] contained tetanus, diphtheria, streptococcus, old tuberculin, Candida, Trichophyton, and Proteus mirabilis antigens and glycerin as a control substance. The antigens were applied on the volar side of the forearm by firm pressure with a mechanical applicator. The skin test was evaluated 48 h after application. The final score consisted of the number of positive antigen reactions and the sum of the mean diameters of these reactions. A reaction smaller than 2 mm was considered negative.

Tetanus antibody assay. Patients were vaccinated against tetanus 2 days prior to operation (Tetanol 0.5 ml)[13]. Specific antibody production was assessed immediately prior to vaccination as well as on postoperative day 7 by using enzyme immunoassay (Enzyquick)[14]. The assay measures the antibody production (IgG) against tetanustoxoid by adding anti-human IgG-peroxidase following incubation of 200 µl patient serum diluted 1:20 with a test system containing tetanus toxoid. The antibody concentration was measured photometrically at 450 nm.

Statistical analysis

Results obtained were analysed statistically with the use of variance analysis for intergroup comparison. Student's paired *t*-test was used for intragroup comparison. Data are given as the mean ± standard error of the mean. A probability value less than 0.05 was considered to be significant.

Results

Clinical results

One patient (group A) died during the early postoperative period of 30 days, for an operative mortality rate of 2.5% of the total patient population studied. Death occurred on postoperative day 10 due to multiple organ dysfunction syndrome following pneumonia which was detected on postoperative day 5. Microbiologic examinations revealed Candida as the infectious agent.

In vitro studies

CD4+ PBMC. In group A patients, the percentage of CD4+ PBMC, primarily representing TH, decreased significant-

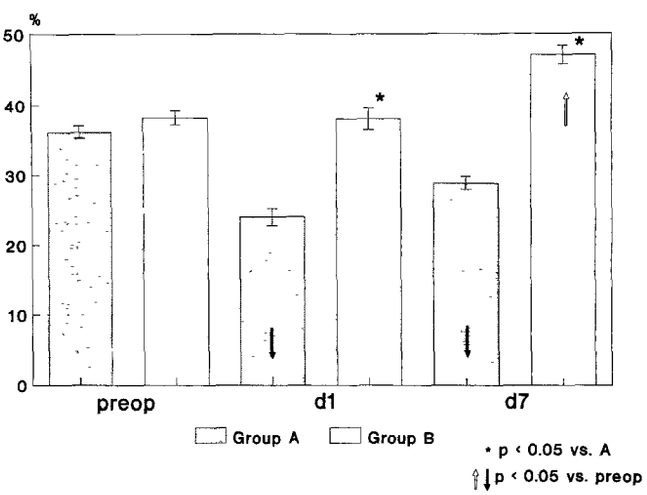


Fig. 2 Percentage of CD4+ peripheral blood mononuclear cells primarily representing helper T cells for groups A and B. *Arrows* indicate a significant difference between preoperative and postoperative results, *asterisks* indicate a significant difference of the results between groups A and B

[10] Gibco, Grand Island, N.Y., USA
[11] Becton Dickinson, Sunnyvale, Calif , USA
[12] Merieux, Hamburg, Germany
[13] Behringwerke, Marburg, Germany
[14] Immuno, Heidelberg, Germany

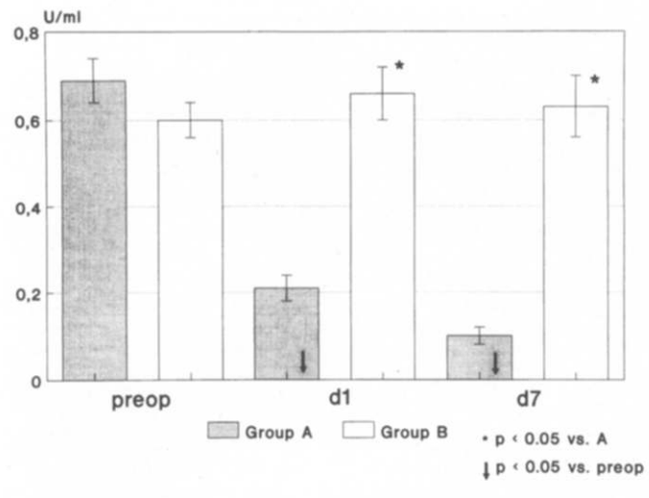


Fig. 3 Interleukin-2 synthesis (U/ml) following peripheral blood mononuclear cell stimulation with phytohemagglutinin for groups A and B. *Arrows* indicate a significant difference between preoperative and postoperative results. *asterisks* indicate a significant difference of the results between groups A and B

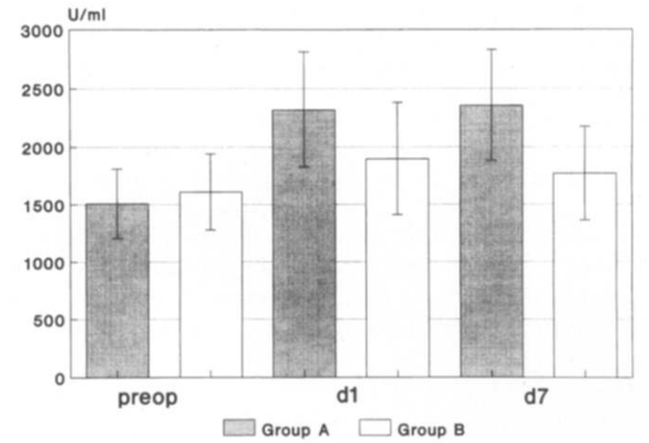
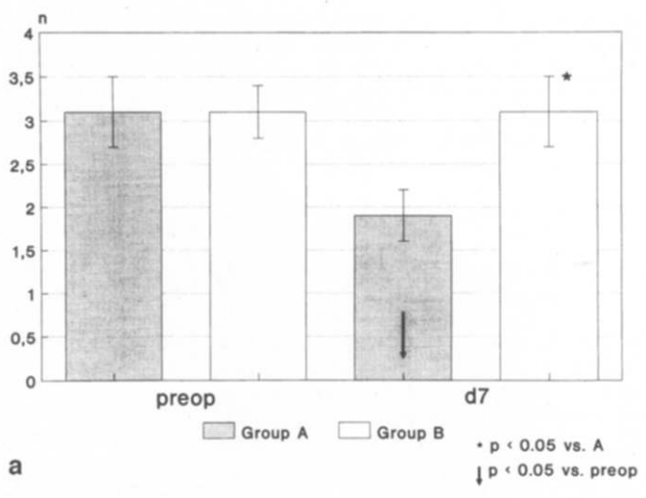


Fig. 4 Interleukin-6 synthesis (U/ml) following peripheral blood mononuclear cell stimulation with phytohemagglutinin for groups A and B



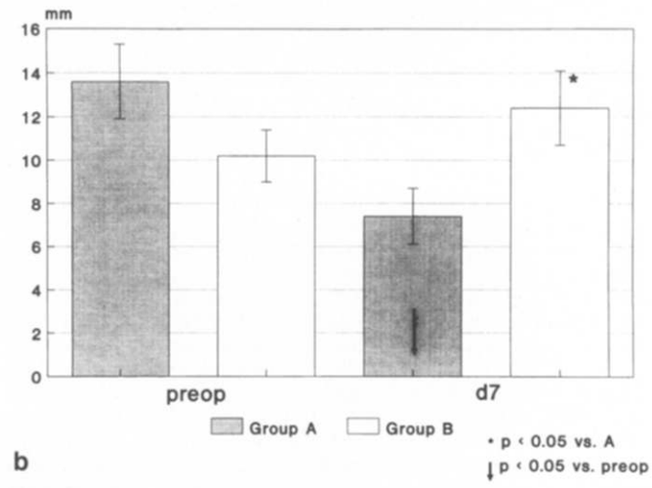


Fig. 5 a Number of positive delayed-type hypersensitivity skin reactions for groups A and B. *Arrows* indicate a significant difference between preoperative and postoperative results, *asterisks* indicate a significant difference of the results between group A and B. **b** Mean diameter of delayed-type hypersensitivity skin reactions for groups A and B. *Arrows* indicate a significant difference between preoperative and postoperative results. *asterisks* indicate a significant difference of the results between groups A and B

ly on the first postoperative day to 24±1.2% as compared to a preoperative baseline value of 36±1%, and remained significantly depressed until the end of the observation period (29±1%). (Fig. 2). In contrast, group B patients showed no change in CD4+ receptor expression on the first postoperative day (38±1.5% vs 38±1%) and even demonstrated a significant elevation on day 7 (47±1.3%), with a resulting statistically significant difference compared to group A on postoperative days 1 and 7.

Interleukin-2 synthesis capacity. The synthesis capacity of PBMC for IL-2 following PHA stimulation was significantly reduced in group A to 32.1±5.6% on postoperative day 1 and 14.6±2.8% on postoperative day 7 as compared to the preoperative baseline values (Fig. 3). In group B patients, postoperative IL-2 synthesis capacity remained within the range of preoperative baseline values (109±7.5% and 108±10.9%). Intergroup comparison showed a significant difference between group A and B in terms of the postoperative results.

Interleukin-6 synthesis capacity. In contrast to the previous results, PBMC synthesis capacity for IL-6 increased in group A to 154±15% on postoperative day 1 and 156±25% on postoperative day 7 (Fig. 4), while it remained nearly unchanged in group B (118±12% and

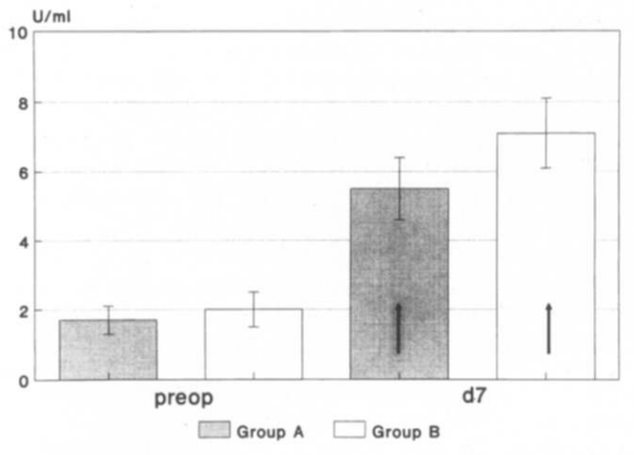


Fig. 6 Specific antibody production (U/ml) following tetanus vaccination for groups A and B. *Arrows* indicate a significant difference between preoperative and postoperative results

110 ± 17%). None of the results showed a statistically significant difference.

In vivo results

Delayed-type hypersensitivity skin response. The number of positive reactions to the antigen skin test battery (Fig. 5a) as well as their mean diameter (Fig. 5b) decreased significantly in group A on postoperative day 7 as compared to preoperative values. The mean number of positive reactions dropped from 3.1 ± 0.4 to 1.0 ± 0.3 and the mean diameter from 13.6 ± 1.7 mm to 7.4 ± 1.3 mm. Group B patients showed nearly no difference between preoperative and postoperative results with the mean number of positive reactions being 3.1 ± 0.3 preoperatively and 3.1 ± 0.4 on postoperative day 7, and the mean diameter being 10.2 ± 1.2 mm preoperatively and 12.4 ± 1.7 on postoperative day 7.

Statistical analysis revealed a significant difference for both the number of positive reactions and the mean diameter on postoperative day 7 between the two groups.

Antibody production. Specific antibody production following tetanus vaccination increased significantly on postoperative day 7 in both groups, from 1.7 ± 0.4 U/ml to 5.5 ± 0.9 U/ml in group A and 2.0 ± 0.6 U/ml to 7.1 ± 1.0 U/ml in group B. The difference in the results between the two groups did not gain statistical significance (Fig. 6).

Discussion

The results of this study suggest that the alterations within CMI observed in previous studies [1, 3, 6, 15] may represent a shift or an imbalance of T-helper cell subsets. A significant reduction of IL-2 synthesis capacity as well as a significantly impaired DTH skin response indicate that TH1 response is depressed in patients following cardiac surgery with extracorporeal circulation [14, 21]. Interleukin-2 is predominantly produced by TH1 and represents a central cytokine for T cell proliferation and differentiation, while DTH skin response represents the prototype of a TH1-mediated immune reaction [14, 16, 21]. Interleukin-6 synthesis capacity and specific antibody production, both representing primarily TH2-mediated events [19], were either unaltered with a trend towards elevated production or showed a normal reaction to an adequate antigenic challenge. This indicates that the TH2-mediated pathway remains nearly unaffected by the immunologic alterations following cardiac surgery. Thus, the reduction in the percentage of CD4+ PBMC, primarily representing T-helper cells, may be caused by an almost exclusive reduction of TH1 without affecting TH2, resulting in an imbalance of TH1 and TH2 subsets.

Since differentiation into TH1 and TH2 cells requires activation of CMI [17], the question remains which stimulus may have activated the CMI response. With the exception of one single patient with pneumonia, there was no clinical evidence for any event other than CPB which may have activated the CMI system in this study. Thus, we may hypothesize that TH cells are primed by CPB to respond with a TH2-mediated reaction to the antigenic challenges produced during CPB. This type of reaction appears to be physiologic, since most of the by-products of CPB represent extracellular antigens which are usually eliminated using the TH2-mediated pathway of CMI response [13, 14, 16–18, 20]. Thus, the immunologic alterations observed in vitro and in vivo in this study could represent a completely adequate response of the CMI system to CPB. This could, in addition, explain the striking differences between immunologic and clinical findings, i.e. serious alterations of immunologic parameters indicating functional changes of the immune system usually interpreted as impairment, but no evidence for adverse clinical effects [1, 3, 6, 9, 15].

However, protection of the TH2 pathway can apparently be achieved only by some form of TH1 depression. Even this depression could be seen as physiologic, since an adequate response to CPB apparently does not require an intact TH1 pathway. The depression of TH1 response may gain clinical significance whenever this type of CMI reaction is needed, i.e. in the case of perioperative systemic infections caused by viruses or bacteria [13, 14, 16–18, 20]. It is known that perioperative systemic infections following cardiac surgery are frequently associated with opportunistic microorganisms and an unusually high rate of multiple organ dysfunction syndrome, subsequently resulting in a high mortality rate [2, 10]. Both indicate some kind of functional impairment within CMI. The hypothesis of an impaired TH1 response, i.e. a significant reduction of one essential cellular element and one essential cytokine re-

quired for immunoreactivity, could explain these clinical observations.

Immunomodulatory intervention with indomethacin and thymopentin could completely normalize the in vitro and in vivo results in this study. Both TH1 and TH2 parameters showed an adequate reaction postoperatively, as compared to preoperative results, with no significant changes of the in vitro parameters, an unaltered DTH skin response and a normal reaction to the antigenic challenge represented by tetanus vaccination. The TH1 pathway of CMI response was therefore protected by immunomodulation, while TH2 response remained unaffected. These immunobalancing effects of both immunomodulatory agents used in this study are in line with previous studies [5, 8, 22]: only major deviations from the baseline values are counteracted, while no effect can be observed on parameters or reactions which remain within a physiologic range.

Some limitations of this study require comment. First, we used two different antibiotics, two different types of oxygenators and cardioplegic solutions and implanted mechanical as well as porcine valve prostheses. All these factors may have influenced the immunologic reactions observed in this study, although they were randomly distributed within the two groups. Second, we could not show that the depression of TH1 response does, in fact, result in an increased susceptibility to opportunistic infections with its adverse effects on clinical outcome. We could not demonstrate that the normalization of in vitro and in vivo parameters of the CMI system implies an improved outcome. Both shortcomings are due to the small number of patients studied. In addition, we do not know if T-helper cells at the site of infection react in vivo in the same way as did the T-helper cells of the circulating blood in vitro in this study, although the in vivo results strongly support the in vitro data. Finally, due to the absence of a technique for identification of TH1 and TH2 subsets, the hypothesis of a TH1/TH2 imbalance was based on the synthesis pattern for two cytokines, which represent only a part of the cytokines produced by TH1 and TH2. Nevertheless the results of this study as well as the data available from the literature [1, 3, 6, 9, 15] indicate

- that the CMI response is substantially altered following cardiac surgery with extracorporeal circulation,
- that these alterations do not necessarily result in adverse clinical effects but may even represent a physiologic reaction,
- that these alterations may gain clinical significance if a certain type of antigenic challenge requires a certain type of CMI response, i.e. a bacterial or viral infection requiring a TH1 response, and finally,
- that these alterations can be normalized by immunomodulation.

Therefore, the results of this study indicate the need for further investigations to identify patients at risk for perioperative infections, who may benefit from immunomodulatory intervention, and to assess the effect of this type of treatment on perioperative mortality and morbidity.

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Discussion

Dr. S. Thelin (*Uppsala, Sweden*). I would just like to know if you have any comment as to the background. What is actually the cause of the changes in the immune system, I mean regarding leukocyte changes during extracorporeal circulation and such changes? If you can speculate a little bit about that.

Dr. A. Markewitz (*Munich, Germany*). We eliminated the quantitative changes of extracorporeal circulation by adjusting the peripheral blood mononuclear cells to a standard number. But I think what we see here is that we operate on older and older patients and their immunoreactivity is much lower than the immunoreactivity of a young patient. So we go into this operative situation with a patient who is immunodepressed because of his age. With ECC we induce some kind of immune response and the patient is able to react to this antigenic challenge by activating this TH2-mediated pathway. But if there is an additional trauma along with cardiopulmonary bypass, he is unable to react to this trauma because he has wasted all of his immunologic capacity in responding to cardiopulmonary bypass in an adequate way. Now he needs help, and I think we have found a way to help the patients. However, further studies are necessary to identify the patients who will need this kind of treatment.

Dr. B. Walpoth (*Bern, Switzerland*). Were your patients operated under hypothermia?

Dr. A. Markewitz: Moderate hypothermia, 28 °C.

Dr. B. Walpoth: The reason why I ask is, as you know, there are now several groups, especially the group in Houston, Texas, doing brain surgery under moderate hypothermia as well, and the one issue which has come out is that the brain protection is better but the patient has significantly more infections, and that might also show that hypothermia on top of cardiopulmonary bypass might induce some immunomodulation with some deficiency, as you showed very elegantly. What is your point on this?

Dr. A. Markewitz: We know that immunologic cells, depending on the type of cells, have a different temperature optimum. The same may apply for the mediators of immune reaction. Therefore, one can speculate that deep hypothermia may inhibit immunoreactivity in some way However, this speculation awaits confirmation by prospective randomized studies.

Dr. R. Landymore (*Halifax, Canada*). In your two previous papers, which were published during the last year, you reported that a combination of indomethacin and synthetic thymic hormone completely reversed the alteration of the immune response and that indomethacin partially reverses the downregulation. Since we use indomethacin frequently to treat post-pericardiotomy syndrome, would you advocate the use of indomethacin after cardiac surgery?

Dr. A. Markewitz: In contrast to my previous papers, I think that indomethacin is not enough, at least in the dosage that we use it, to prevent what the clinicians now call SIRS, and that is what we try to do with indomethacin or with cortisone. I think if you are in a clinical situation where you need a lot of help, that means a critically ill patient in the intensive care unit, I now prefer to use hydrocortisone. For the routine patient you can use indomethacin if you think about two facts: the first is that you have to have a good gastric protection, and, second, indomethacin adversely affects kidney function.

Mr. J. Murday (*London, England*). I was a little bit surprised that the effect on cell-mediated immunity from the operation whether it be the cardiopulmonary bypass, the temperature or some other factor, would have lasted so long Can you give us some idea from your work how long-lasting the effect of cardiac surgery is on depressing cell-mediated immunity.

Dr. A. Markewitz: Well, due to logistic problems, we never studied patients beyond day 10, and even at that day we could see some, non-significant effects on cell-mediated immunity. So I guess depending upon the age of the patient and the preoperative immunologic status, it will take an average patient 1–3 weeks to recover completely from cardiac operations. I am aware that this differs from the experience of others, which have been published in recent years. But if you look carefully at these papers, you will find that they investigated much younger patients, and I guess age is a key point when looking at immunoreactivity following cardiac surgery.