Immunomodulatory Therapy With Thymopentin and Indomethacin

Successful Restoration of Interleukin-2 Synthesis in Patients Undergoing Major Surgery

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Prostaglandin E_2 (PGE$_2$)-mediated monocyte (Mϕ) suppressor activity and inadequate T-helper cell function represent the mechanistic keystones of trauma-induced impairment of cell-mediated immunity (CMI). In a prospective randomized trial, the immunorestorative potential of a combined therapy with the thymomimetic substance Thymopentin (TP-5; Timunox®, Cilag GMBH, Sulzbach, FRG) and the cyclooxygenase inhibitor indomethacin (Indo) in 60 patients (mean age, 63 ± 2 years) undergoing open heart surgery was studied. Perioperative immunologic screening was carried out on days −2, 3, 1, 5, and 7 and included the *in vivo* delayed type hypersensitivity (DTH) skin response, phenotyping for peripheral blood mononuclear cell (PBMC)-specific and nonspecific induction of lymphoproliferative responses, *in vitro* interleukin-2 (IL-2) synthesis, as well as the serum concentration of D-erythro-Neopterin (NPT) and of gamma interferon (γ-IFN). The study protocol comprised three groups (n = 20): PA (Indo 150 mg administered intravenously on days 0 to 5), PB (TP-5 administered subcutaneously on days 0, 2, 4, and Indo), and PC (control). In contrast to PC, significant immunorestoration could be demonstrated in PB, as DTH scores on day 7, as well as proliferative responses in cell cultures were not depressed after operation (p < 0.05). Cell-surface receptor expression for the CD3+, CD4+, and IL-2 receptor-positive (IL-2R+) lymphocyte subpopulations following surgery was reduced to 75% of baseline values in PC, while in PB, receptor protection for CD4+ and IL-2R+ subpopulations (more than 15% above baseline) was observed. Interleukin-2 synthesis (average baseline value, 0.7 + 0.08 U/mL) in cell cultures of PC was massively suppressed, with lymphokine concentrations in the supernatants never more than 0.27 ± 0.05 U/mL. In PA cultures, IL-2 synthesis was impaired as well but not as precipitously as in PC. In contrast, in PB cultures, the average IL-2 production on consecutive postoperative days was never below baseline values. This study clearly demonstrates that the combined Indo/TP-5 therapy is superior to single Indo administration and can ade-

quately preserve and/or restore intact MϕT-cell interaction and thus appears to be a feasible approach to maintain normal host defense activity in traumatized individuals.

PATIENTS UNDERGOING CARDIAC surgery with extracorporeal circulation (ECC) are subjected to an immediate massive impact on their host defense integrity due to the combined effect of tissue trauma, multiple blood transfusions, and a whole-body inflammationlike state, induced through the extensive contact between blood and foreign material. The resulting impairment of cell-mediated immunity (CMI) has its clinical correlate because there is enhanced susceptibility of these patients to infectious complications.[1-3] In our own institution, during the year 1990, 55% of the deaths in patients undergoing ECC were due to septic multiple-organ failure.

For the reasons outlined above, it can be suggested that the major cardiac surgery patient can serve as an excellent *in vivo* model to investigate the efficacy of therapeutic strategies designed to counteract the impairment of immunomechanistics induced through severe trauma. We and others have demonstrated[4-6] that the alteration of CMI following trauma is mainly due to the disruption of intact monocyte (Mϕ)/T-cell interaction. Within this phenomenon we see a shift of the cell ratio in the compartment of peripheral blood mononuclear cells (PBMC), with a considerable increase of prostaglandin E_2 (PGE$_2$) synthesizing Mϕ and a simultaneous decrease of functionally competent CD3+ and CD4+ lymphocytes. T-cell dysfunction in states of profound stress is characterized by impaired synthesis of two crucial cytokines—in-

terleukin-2 (IL-2) and gamma interferon (γ-IFN).[7,8] The inability to produce adequate amounts of IL-2 results in incomplete proliferative T-cell responses to antigenic stimuli, while a lack of γ-IFN results inefficient Mφ antigen presentation. It has been demonstrated that both defects are keystones of suppressed CMI function following trauma, with subsequent development of sepsis.[9-11]

The information derived from the dissection of down-regulatory mechanisms responsible for the development of injury-related immunoincompetence provided the incentive for the development of therapeutic regimens designed to prevent a major collapse of CMI.

The use of two classes of substances seemed to be most suitable for immunoprotection and/or immunorestoration: first nonsteroidal anti-inflammatory drugs, which block immunoreactive PGE_2,[12] the common link of malfunction of the Mφ/T-cell interactive network in states of trauma; and second the synthetic thymomimetic pentapeptide Thymopentin (TP-5; Timunox®, Cilag GMBH, Sulzbach, FRG), with its crucial characteristics—restoration of immunobalance, T-cell activation, and acceleration of T-cell recruitment.[13]

We have conducted a number of clinical trials in recent years to scrutinize the immunoaugmenting potential of TP-5 as well as of the cyclooxygenase inhibitor indomethacin (Indo) in patients undergoing major surgery.[14] A perioperative two-shot subcutaneous administration of 50 mg TP-5 in patients undergoing open heart surgery resulted in a restoration of the in vitro lymphocyte proliferative responses, as well as in the delayed type hypersensitivity (DTH) responses, compared to a placebo-treated control population. It was disillusioning to recognize, however, that T-cell receptor protection (CD3+, CD4+) and restoration of IL-2 synthesis could not be achieved with that treatment modality.[15]

Conversely postoperative administration of Indo in patients undergoing gastrectomy or reconstruction of the abdominal aorta resulted in an impressive protection of T-cell receptor expression for the CD3+, CD4+, and IL-2 receptor-positive (IL-2R+) subpopulations. This treatment also controlled overwhelming relative monocytosis. Furthermore the preservation of the preoperative in vivo DTH immunoreactivity, in contrast to untreated patients, could be demonstrated.[16] However restoration of depressed IL-2 synthesis could not be attained with Indo administration, which was in contrast to many in vivo experiments that showed that Indo restores depressed IL-2 production.[17]

Based on the findings in these single-agent studies and the knowledge that trauma induced depression of CMI represents a multimechanistic phenomenon, the protocol for a combined-agent therapeutic trial was designed. It was the objective of this prospective randomized study to

quantify, specify, and compare the immunorestorative potential of a combined therapy with the cyclooxygenase inhibitor Indo and the thymomimetic substance Thymopentin versus single-drug administration of Indo following ECC.

Cell-mediated immunity parameters studied, in vivo and in vitro, included the DTH skin response to recall antigens, PBMC phenotyping, specific and nonspecific induction of lymphoproliferative responses, and in vitro IL-2 synthesis. In addition, for CMI serum markers, we evaluated the concentration of D-erythro-Neopterin, a sensitive indicator of Mφ activation, as well as the concentration of γ-IFN as a marker for T-cell activation. The results indicate that simultaneous cyclooxygenase inhibition and T-cell activation greatly enhanced the forward regulatory axis of cell-mediated immune mechanisms following ECC.

Materials and Methods

Patient Population

Surgical patients in the surgical wards and the intensive care unit of the Department of Cardiac Surgery of the LM University Munich, Klinikum Großhadern, were studied. This study was approved by the Medical Ethics Committee of the Faculty of Medicine.

From November 1, 1989 until October 31, 1990, studies were conducted in 60 patients (45 men, 15 women) whose average age was 63 ± 7 years. All patients had acquired or congenital heart disease and had to undergo ECC surgery with coronary artery bypass grafting or valve replacement (Table 1).
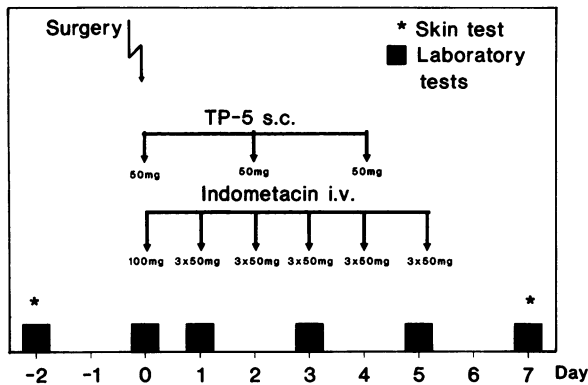
Experimental Protocol

For the prospectively randomized study (Fig. 1), patients were divided into three groups: group A patients (PA) (n = 20) were given indomethacin (Confortid®, Dumex, Denmark) in 100-mg intravenous doses immediately after surgery (D0), and 500-mg doses three time daily until day 5 after operation. Group B patients (PB) (n = 20) besides Indo therapy also received 50 mg of TP-5 (Tim-

TABLE 1. Clinical Data (n = 60)

Characteristic	
Average age (yr)	63 ± 7 (range, 55–78)
M/F	45/15
Surgical procedure	
CABG	n = 46
Valve replacement	n = 14

Experimental Protocol

FIG. 1. Depiction of the experimental protocol, showing time of indomethacin and TP-5 administration and frequency of laboratory tests.

munox®, Cilag, FRG) administered subcutaneously 2 hours before operation and 48 hours and 96 hours after operation. Group C patients (PC) (n = 20) served as the control population, undergoing conventional intensive care unit therapy after operation.

Age, underlying disease, and quality of surgical procedure were highly comparable in all three groups.

Immunologic screening of the patients was carried out twice before operation, immediately after admission, and on the day of the operation, but was calculated as one preoperative value, as well as on days 1, 3, 5, and 7 after operation.

Cell Preparation and Culture

For lymphocyte studies 50 mL of peripheral blood was obtained in sterile heparinized tubes. The blood samples were diluted 1:2 in Hanks' buffered saline solution (Gibco Laboratories, Grand Island, NY) with 2% Penicillin-Streptomycin. Peripheral blood mononuclear cell isolation was performed immediately by standard Ficoll-Hypaque density gradient centrifugation at 1500 rpm at 4 C for 35 minutes. After resuspending the cells with 15% fetal calf serum (Gibco Laboratories), cell counts were performed with a hemocytometer using trypan blue exclusion as a test of viability. Viability always exceeded 95%. Appropriate cell suspension concentrations were then prepared for the different assays to be performed.

Mitogen Assay

At concentrations of 1 × 10⁵ cells per well, PBMCs were added in triplicates to flat-bottomed, 96-well microtiter plates and antigen cocktail (AgC) was added to the wells at a final concentration of 50 mg/L. Antigen cocktail

(Behring Co., Marburg, FRG) is a mixture containing five antigens—purified protein derivative, tetanus toxoid, streptolysin, mumps, and vaccinia antigen.

The cultures were incubated at 37 C in a 6% CO_2 incubator for 120 hours. Six hours before harvesting, the cultures were pulsed with 18.5×10^3 Bq per well of tritiated thymidine. The cultures were harvested on glass-fiber paper with a multiple-automated sample harvester. Vials, holding the filter strips and scintillation fluid, were counted in a beta counter. The net count per minute of triplicate cultures was calculated as the net count for the cells with AgC minus the net count for the cells without AgC. Lymphocyte proliferation was also performed with phytohemagglutinin (PHA), which was added to the triplicates at a final concentration of 0.5 $\mu g/mL$. These cultures were incubated for 72 hours before being pulsed with thymidine.

PBMC Phenotyping

Phenotyping of Ficoll-Hypaque preparations of mononuclear cells, as described elsewhere,[18] was performed using monoclonal antibodies to quantitate CD3+, CD4+, and CD8+ T cells (Becton-Dickinson, Heidelberg, FRG), LeuM3+ monocytes (Becton-Dickinson), as well as IL-2R+ cells (Biotest Diagnostics, FRG). The number of cells that were stained with the antibodies was assayed using fluorescence microscopy. The IL-2R antibody identifies the human IL-2R on mitogen- and antigen-activated T cells. Enumeration was performed after 48 hours incubation of the PBMCs with 1 mg/mL PHA.

IL-2 Generation and Activity Assay

Interleukin-2 was generated by culturing PBMC suspensions at a cell concentration of 20 PBMCs per liter in 5-mL culture tubes in the presence of highly purified PHA at a final concentration of 2.5 mg/L at 37 C, 6% CO_2. After 48 hours supernatants were collected and stored at −70 C until assayed.

The assay for the detection of IL-2 activity was a modification of the method described by Gillis et al.[19] Briefly frozen long-term cultures (5 days) of human T cells with concanavalin A blasts serving as IL-2–sensitive target cells were thawed, washed, and resuspended in Roswell Park Memorial Institute 1640 medium supplemented with 2% streptomycin-penicillin and 20% pooled human serum. Supernatants to be tested for the amount of IL-2 released on stimulation with PHA were placed in 100-μL serial dilutions from 1:2 to 1:128 in 96-well, round-bottomed microtiter plates in triplicate. Next 100 μL of concanavalin A blasts were added to each well, resulting in a final concentration of 4 × 10⁴ cells per well. The IL-2 activity of the supernatants to be tested was compared with the ac-

tivity induced by the IL-2 standard solution, which contained 1 U/mL by definition (Lymphocult HP, Biotest, Offenburg, FRG). Units of IL-2 were calculated by comparison with the cell proliferation induced with the Lymphocult test solution.

Assay for γ-IFN

Immunoradiometry was used (a gift of Centocor Inc., Malvern, PA) employing a modified application[20] that allows the detection of γ-IFN in sera with sufficient sensitivity. Beads with monoclonal antihuman γ-IFN antibody were incubated with 200 μL serum at room temperature for 16 hours. Then the beads were washed with 3 mL distilled water and incubated for an additional 16 hours with 200 μL of ^{125}I-labeled tracer. With this modified procedure, more than 80% of the analyte is bound to the solid-phase antibody.[20] Radioactivity was counted with a gamma counter (CliniGamma 1272; Wallac Oy, Turku, Finland). Gamma interferon activity is expressed as National Institutes of Health (NIH) units (U).[21] The limit of detection was 18 U/L.[20]

Assay for Neopterin

Neopterin was quantified by radioimmunoassay (RIA-cid, Henning-Berlin, Berlin, FRG). Fifty microliters of serum were incubated with 100 μL of neopterin antiserum for 1 hour at room temperature, and 100 μL of ^{125}I-labeled tracer was added followed by incubation for 1 hour. Two milliliters of aqueous polyethylene glycol 6000 solution (60 g/L) was then added. After centrifugation at 2000g for 10 minutes, radioactivity was counted with the gamma counter. The detection limit was 1 nmol/L.[22]

DTH Skin Testing

The DTH response to seven recall antigens was carried out 2 days before surgery and on day 7 after operation. The recall antigens (tetanus, diptheria, Streptococcus, old tuberculin, Candida, Trichophyton, and Proteus mirabilis) and a glycerin control are contained in a test system (Multitest Mérieux, Hamburg, FRG). The antigens were applied on the volar side of the forearm by firm pressure with a mechanical applicator. The skin test was evaluated 48 hours 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.

Statistical Analysis

Statistical analysis was carried out using analysis of variance for intergroup comparison. Student's paired t test was used for intragroup comparison. Probability values less than 0.05 were considered significant.

Results

Phenotyping Studies

A significant persistent reduction of cell-surface receptor expression for the CD3+, CD4+, and IL-2R+ subpopulations following surgery compared to preoperative values was observed in PC (Fig. 2). The lowest level of depression was seen on day 1, with an average range of reduction for CD3+ and CD4+ leukocytes between 20% and 30%. Indo treatment resulted in a clearly demonstrable alleviation of depressed receptor expression in PA compared to PC. In PB, after day 1, an absolute receptor protection, partially with elevations toward supranormal ranges, especially for the CD4+ subpopulation 114% ± 4% (D3), 120% ± 4% (D5), 124% ± 3% (D7), was observed.

An adequate number of IL-2R+ (CD25+) cells could be maintained with combined therapy, as well as with sole Indo treatment, compared to a considerable receptor depletion in the PC group.

The baseline value of 14% ± 0.6% CD8+ cells was elevated on day 1 in all groups but returned to normal in PA and PB on day 7, in contrast to PC. When calculating the CD4+/CD8+ ratio on consecutive postoperative days compared to the average baseline value of 2.8 ± 0.2 for all groups, a clear gradation of values for the individual groups with crucial differences between PC versus PA and PB was observed. A striking difference appears on day 7 with a CD4+/CD8+ ratio (2.0 ± 0.1) in PC, which is still below baseline (p < 0.05), while there was a value (3.7 ± 0.2) for PB, expressing a significant overcorrection from baseline (p < 0.05)

Surgery resulted in a massive initial increase of CD14+ Mφ in all groups compared to the average baseline value of 15% ± 0.7% cells. On day 1 the number of Mφ within the PBMC population calculated ranged between 36% ± 2% (PC), 31% ± 2% (PA), and 28% ± 1% (PB). A parallel pattern of decreasing numbers was seen on consecutive days after surgery for all groups. However the initial significant differences in the Mφ counts in the postoperative intergroup comparison persisted until the end of the observation period. Thus on days 5 and 7 there was still a +61% ± 9% and +46% ± 9% elevation in PC, while in PA (+34% ± 9% [day 5], +13% ± 7% [day 7]), and PB (+29% ± 9% [day 5], −1.5% [day 7]) the counts were significantly lower.

Proliferative Responses

The preoperative average PHA-induced lymphocyte proliferation in all patient groups (31892 ± 1497 CPM [PC], 33159 ± 1504 CPM [PA], and 31548 ± 1412 CPM [PB]) was nearly identical. Furthermore the AgC-induced proliferative responses were similar: 17670 ± 605 CPM (PC), 18473 ± 719 CPM (PA), and 17503 ± 396 CPM (PB) (Fig. 3).
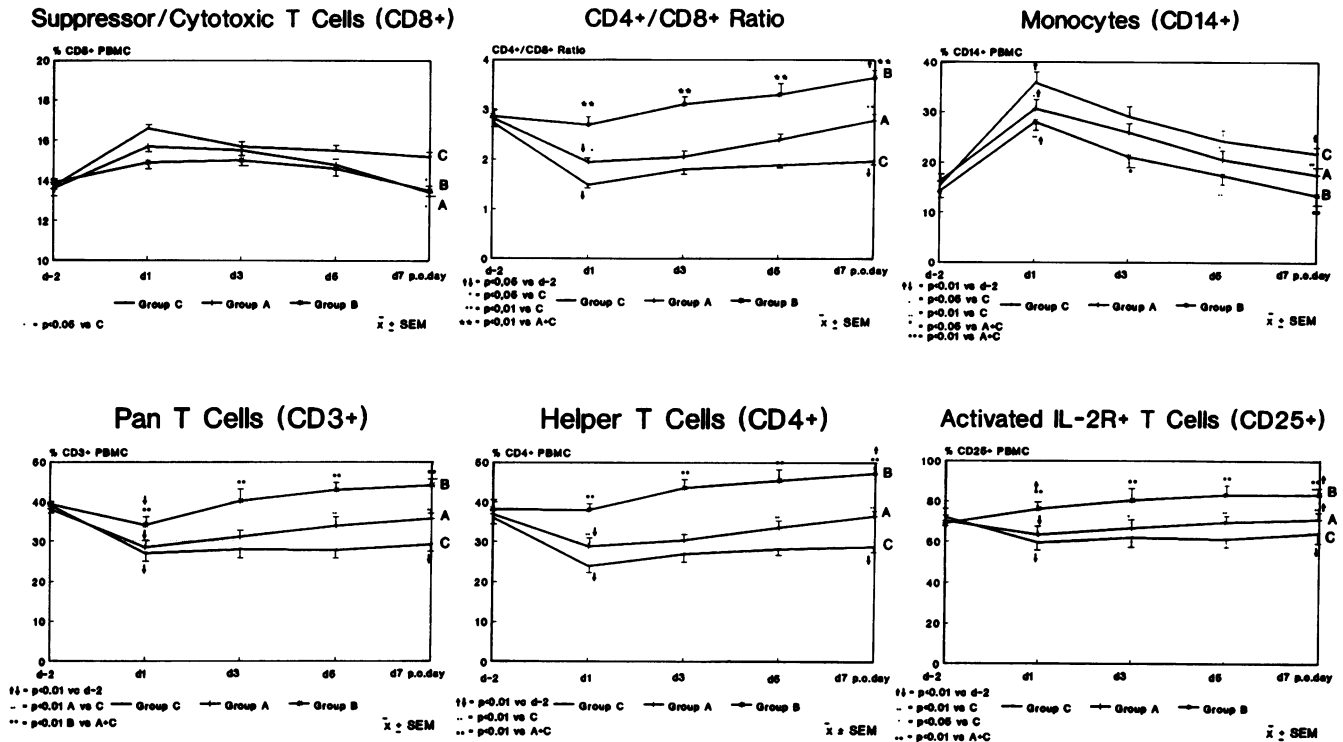
PBMC Phenotyping

FIG. 2. Phenotyping of peripheral blood mononuclear cell subpopulations on consecutive postoperative days, for the individual patient groups PA, PB, and PC. Changes in CD3+, CD4+, CD8+, and IL-2 R+ (CD25+) T cells, in the CD4+/CD8+ ratio and in CD14+ monocytes, marked with the monoclonal antibody LeuM3 are depicted. Asterisks indicate values that are significantly different ($p < 0.01$, $p < 0.05$) in intergroup and intertime (vsD-2) comparison.

PHA-induced proliferation in PB cultures was not depressed during the postoperative course, but showed mild elevations within the range of +17.8% (on day 3) and +23.6% (on day 5) compared to baseline.

In contrast proliferation values of PC and PA cultures were significantly suppressed ($p < 0.05$), reaching 58.2% ± 2.6% (PC) and 74.9% ± 2.3% (PA) of baseline levels.

For PC and PA, although a gradual recovery of proliferation was seen, the responses were still significantly lower on day 7 compared to preoperative values. There was also a significant difference between the proliferative responses of PA and PC on all postoperative days.

Compared to the average baseline values of specific antigen (AgC)-induced PBMC proliferation, a significant decrease on day 1 in all groups was observed. While PB proliferation was reduced to 84.3% ± 3.4% of baseline, PA (67.2% ± 2.9%) and PC (53.4% ± 3.9%) values decreased more substantially. There was a clear tendency toward normalization on consecutive days after surgery in PA and PC, without reaching the baseline level by day
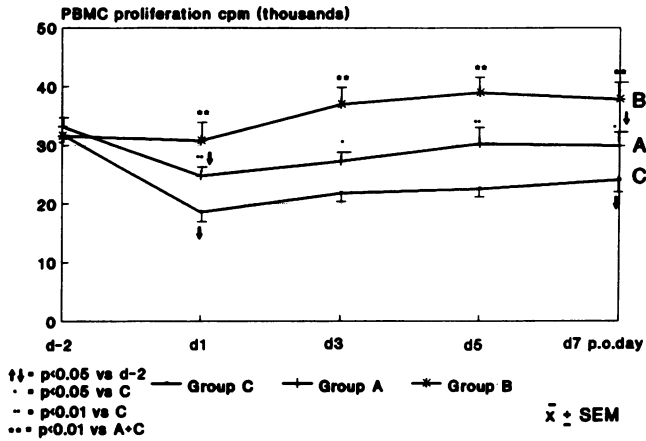
7 ($p < 0.05$). In contrast proliferation of PB on day 5 was already up to 103%.

Interleukin-2 Synthesis

The preoperative values of PHA-induced IL-2 synthesis were comparable in all patient groups with 0.69 ± 0.05 (U/mL) (PC), 0.59 ± 0.04 (U/mL) (PA), and 0.60 ± 0.04 (U/mL) (PB) but were considerably lower than the control value (0.80 ± 0.04 U/mL) derived from healthy human volunteers (Fig. 4).

During the postoperative course, IL-2 synthesis in cell cultures of PC were massively suppressed, with lymphokine concentrations in the supernatants never more than 0.27 ± 0.05 U/mL (on day 3) with a nadir on day 7 (0.10 ± 0.02). In PA cultures a considerable impairment of IL-2 production compared to preoperative values was observed, although it was not as precipitous overall, as in PC. In PA cultures on day 7, the IL-2 production was as low as 29% (0.16 ± 0.02 U/mL) compared to preoperative values. In contrast in PB cultures the average IL-2 pro-

PHA induced lymphocyte proliferation

PBMC proliferation cpm (thousands)

† ‡ • p<0.05 vs d-2
• • p<0.05 vs C   —— Group C   —+— Group A   —*— Group B
- • p<0.01 vs C
••• p<0.01 vs A+C   x̄ ± SEM

AgC induced lymphocyte proliferation

PBMC proliferation cpm (thousands)

† ‡ • p<0.05 vs d-2
• • p<0.05 vs C   —— Group C   —+— Group A   —*— Group B
- • p<0.01 vs C
• • p<0.05 vs A+C
•• • p<0.01 vs A+C   x̄ ± SEM
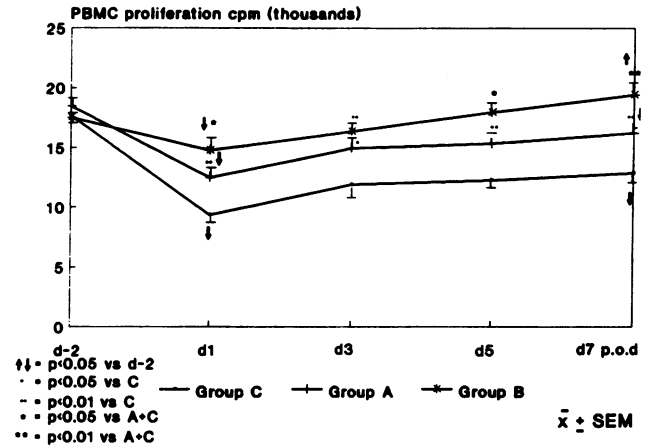
FIG. 3. Nonspecific-, PHA mitogen-induced, as well as specific antigen (AgC)-induced lymphocyte blastogenesis before operation (D-2) as well as on consecutive days after surgery for the three patient groups, is depicted. Asterisks indicate values that are significantly different (p < 0.01, p < 0.05) in the intergroup and intertime (vsD-2) comparison.

duction on consecutive postoperative days was never less than the baseline value, showing even slight elevation up to +18% (0.70 ± 0.08 U/mL) on day 5. On all days there was a significant difference between the IL-2 synthesis in PB versus PA and PC.

DTH Response

In PC the operative trauma resulted in a significant depression of quantity and quality of the skin responses on day 7 compared to the preoperative scores (Fig. 5).

Thus the average value of positive Ag reactions (3.1 ± 0.04) (100%) and the sum of mean diameters of the skin response (13.6 ± 1.4) (100%) decreased to 1.9 ± 0.3 (62% of baseline) and 7.4 ± 1.3 (54% of baseline), respectively. In PA the baseline values (3.0 ± 0.3+ reactions/ 11.4 ± 1.4 mean diameters) decreased to 2.4 ± 0.3 (80%) and 8.5 ± 1.4 (74%), respectively. In comparison to PC, PB values on day 7 were significantly higher (p < 0.05) (3.1 ± 0.4 [100%]/12.4 ± 1.7 [122%]) and did not fall

Interleukin 2 Synthesis

IL-2 [U/ml]

† ‡ • p<0.01 vs d-2   —*— Group C   —◇— Group A   —△— Group B
- • p<0.05 vs C
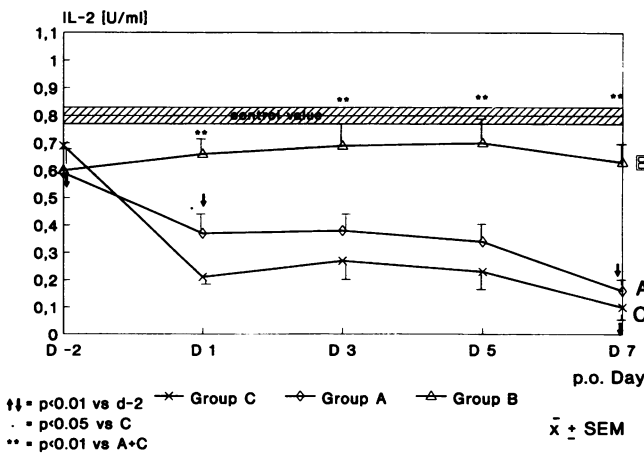•• • p<0.01 vs A+C   x̄ ± SEM

FIG. 4. PHA-induced in vitro IL-2 synthesis before operation (D-2) and on various days following operative trauma for the three patient groups studied. Horizontal bars express the IL-2 control value (0.8 ± 0.04 U/ mL) in this laboratory derived from healthy laboratory volunteers. Asterisks indicate values that are significantly different (p < 0.01, p < 0.05) in the intergroup and intertime (vsD-2) comparison.
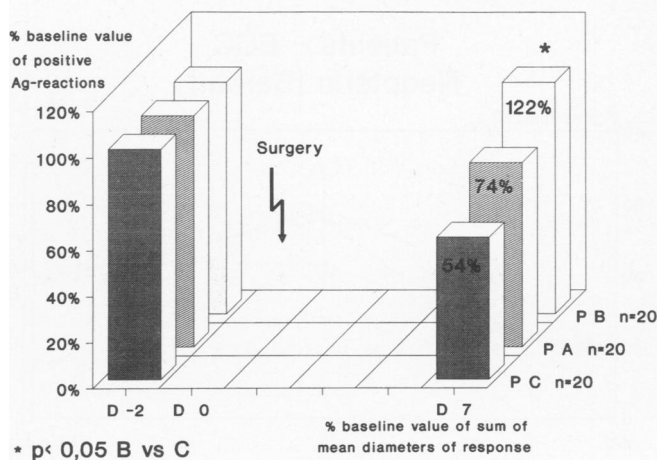
DTH - Skin Response

* p< 0,05 B vs C

FIG. 5. DTH response skin testing was done before operation (D-2) and on D7 after operation. The score of the DTH response consists of the number of positive Ag reactions and the sum of mean diameters of the response. The different height of the individual bars indicates changes in the number of positive Ag reactions after operation. The percentage written on the bars indicate the changes of the mean diameters of response in the individual groups compared to baseline values.

short of their respective baseline values (3.1 ± 0.3/10.2 ± 1.2).

Serum Neopterin

The average preoperative NPT concentration in the patient population studied was 8.9 ± 0.9 nmol/L (nanomolar) (Fig. 6). In PC a very rapid increase of NPT in serum was seen on day 1 (20.8 ± 3.3 nmol/L), with a peak value of 29.1 ± 3.2 nmol/L on day 3. In PA (12.8 ± 6.8 nmol/L) and PB (14.2 ± 6.6 nmol/L) there was a much more subtle increase of this monocyte activation marker. On day 3 the NPT concentration in PA (27.6 ± 4.6 nmol/L) was almost identical to the peak value of PC, while in PB a concentration of about 21 nmol/L was measured, remaining at this level until day 5.

On day 7 NPT concentrations in all patient groups decreased to a level of about 19 nmol/L.

Serum γ-IFN

Serum γ-IFN concentrations in PB were continuously increasing from their preoperative day 2 value of 115 ± 15 U/mL on consecutive postoperative days to 129 ± 21 U/mL on day 1, 144 ± 28 U/mL on day 3, and 143 ± 17 U/mL on day 5, finally reaching 173 ± 49 U/mL on day 7 (Fig. 7). In contrast to these findings, the γ-IFN concentrations on day 1 in PA (105 ± 15 U/mL) and PC (103 ± 13 U/mL) remained within the range of their preoperative levels, then declined in parallel on day 3 to 82 ± 13 U/mL (PC) and 90 ± 11 U/mL (PA), respectively. While until day 7 in PA the preoperative mediator level was regained, γ-IFN concentrations in PC showed a fur-

Patients - ECC
Neopterin [Serum]
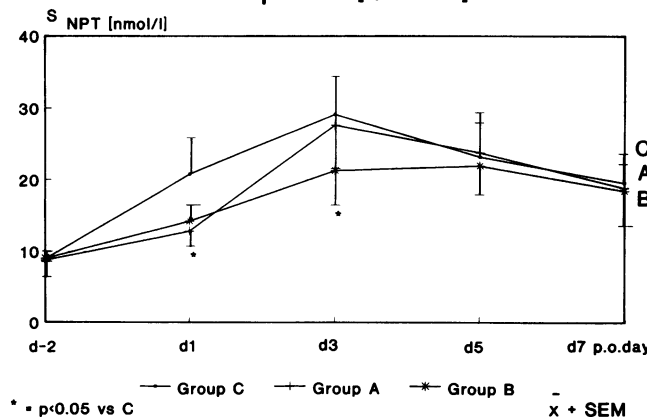
FIG. 6. Perioperative changes of serum NPT levels (nmol/L) in the individual patient groups. Asterisks denote values that are statistically different (p < 0.05) in the intergroup comparison.
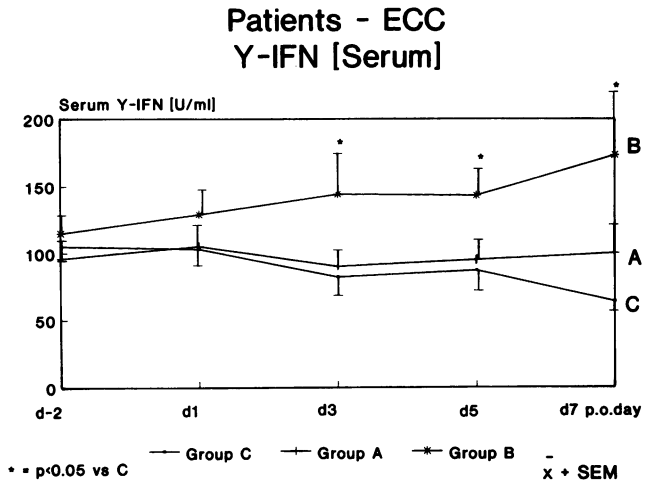
Patients - ECC
Y-IFN [Serum]

FIG. 7. Depiction of perioperative changes of serum γ-IFN levels (U/mL) in the individual patient groups. Asterisks denote values that are statistically different (p < 0.05) in the intergroup comparison.

ther decrease to 64 ± 5 U/mL. The difference of γ-IFN serum concentrations between PC and PB, starting on day 3, were significant (p < 0.05).

Clinical Results

In this population of 60 patients, seven infectious complications (11.6%) were seen during the postoperative observation period of 20 days (Table 2). In group A there were two cases of major infectious episodes with clinical manifestation on day 7 (sepsis of unknown origin) and day 15 (pneumonia). The latter patient died with septic multiple-organ failure on day 20. In group B three infectious episodes became manifest. The origins of infection were the contamination of a central venous line, sternal osteitis, and cystitis, with no infection occurring before day 7.

In group C two infectious episodes were documented. In one patient sternal osteitis occurred on day 5. The second patient died from septic multiple-organ failure originating from pneumonia with clinical manifestation on day 5.

TABLE 2. Clinical Results: Infections (n = 7)

Group A n = 2	Group B n = 3	Group C n = 2
Sepsis (D7)	Cystitis (D8)	Sternal osteitis (D5)
Pneumonia (D15)†	Sternal osteitis (D7)	Pneumonia (D5)†
	Central venous catheter (D7)	

† Died.
D, day.

Discussion

This study shows clearly that combined administration of the cyclooxygenase inhibitor Indo and the thymomimetic drug TP-5 can optimally prevent the trauma-induced depression or breakdown of CMI mechanisms that normally occur in individuals after major operative and ECC injury. While corroborating some previous data derived from single-drug studies with these particular substances, this investigation, through a number of parameters, demonstrates the superiority of the immunoaugmenting effect of a combined Indo/TP-5 administration *versus* single-drug use.

The global assessment of *in vivo* (DTH) immunoreactivity in PB (Indo + TP-5) after operation indicated no difference from the preoperative status, in contrast to a significant reduction seen in PC (no immunomodulatory treatment), while there was a 20% to 25% reduction of the preoperative DTH score in PA (Indo). T-cell receptor (CD3+, CD4+, IL-2R+) protection appeared to be excellent in PB during the postoperative phase, with a tendency toward overcorrection for the CD4+ and IL-2R+ subpopulations on day 7, while simultaneously, under this respective regimen, the counterregulatory activity to limit excessive monocytosis seemed to be most effective. Although Indo administration could alleviate the quantitative unbalances of PBMC subpopulations seen in PC, it could not continuously induce significant improvement as demonstrated in a recent study[16] that investigated different types of operative trauma (gastrectomy, abdominal aortic grafting).

Postoperative depression of lymphocyte blastogenesis was also most sufficiently counteracted with combined TP-5/Indo therapy, which clearly worked better than the two-shot sole TP-5 administration, as demonstrated in a previous investigation in an identical operative model.[15] In that study with Indo, the impairment of proliferative responses was also significantly reduced.

In vitro IL-2 synthesis in cell cultures of PB on consecutive postoperative days did not show any suppression and the values were always the same or more than the baseline level. To our knowledge these findings represent the first report of a successful intervention to preserve adequate T-cell capacity for IL-2 production in humans after trauma.

The serum levels of γ-IFN in the patient population treated with Indo/TP-5 were continuously increasing on consecutive postoperative days, with an average increase of +50% above baseline between days 3 and 7. The increased amount of γ-IFN released in PB provides evidence for the complex efficacy of T-cell activation, triggered through that specific immunomodulatory treatment. Gramma interferon serum levels in PA were within the

baseline range on day 7, in contrast to an average reduction of 35% compared to preoperative levels in PC on day 7.

D-erythro-Neopterin, an indicator of Mφ activation,[23] increased more rapidly in PC within the first 72 hours after trauma compared to PB. By day 7 after operation, NPT concentrations were identical in all three patient groups.

In more recent subsequent tests within this investigation, we analyzed the supernatants of LPS-stimulated PBMC cultures of PA, PB, and PC for the proinflammatory cytokines IL-1, IL-6, and tumor necrosis factor (TNF) (results not shown here). The results showed that interleukin-1 (IL-1) synthesis in PB cultures was significantly higher compared to PA and PC. When including these findings into the body of information derived from this study, one can find striking evidence for the salutary mechanism of action induced by the Indo/TP-5 treatment.

Simultaneous PGE$_2$ blockade *via* Indo and T-cell activation *via* TP-5 apparently represent an ideal strategy to provide protection for the successful development of an immune response for which several levels of control are necessary. Essential steps within the forward-regulatory immune response pathway include: synthesis and release of IL-1 from Mφ, intact Mφ function, intact T-cell receptor function, a sufficient number of functionally intact T-helper cells, IL-2 synthesis and adequate IL-2 release, as well as IL-2-sensitive responder cells with intact capacity of IL-2 receptors.[24] Furthermore Mφ participation can only be sustained *via* γ-IFN production by activated T cells. The downregulation of CMI responses occurs mainly due to PGE$_2$, a regulatory mediator released from inhibitory monocytes, which are released in large amounts after injury. One of the most prominent PGE$_2$ immunoregulatory activities consists of the regulation of IL-2 synthesis.[25] Mediators such as PGE$_2$, which are associated with both specific and nonspecific immunosuppression, have been demonstrated to impinge on the prostaglandin cyclic AMP pathway and it has been postulated that agents that interfere with these mechanisms may counter the immunosuppressive influences. Thus it has been recognized that an immunopharmacologic attack on T-cell disturbances in trauma should either contrasuppress the suppressor-PGE$_2$ by blocking cyclic AMP, or it should have immunorestorative capacities in terms of promoting the cyclic GMP, as for example, thymomimetic substances such as TP-5 do.

Based on these reflections it appears logical that a treatment regimen that combines blocking, ideally with enhancing action, should consist of a combination of immunopotentiating agents that complement each other functionally. Maghsudi et al.,[26] in an animal model of burn injury, demonstrated that the restoration of de-

pressed secondary immune responses to sheep red blood cells could be best accomplished through the additive effect of Indo and TP-5.

Our results demonstrated that using the combination of TP-5 and Indo, all essential components within the lymphokine cascade circuit could be either protected or stabilized after ECC.

When interpreting these data, however, one must stay away from oversimplification, although a dissection of the mechanisms is warranted. It will always remain difficult to demonstrate the absolute causality between a particular agent and a seemingly corresponding function. Many of the findings in this study indeed represent the result of amplification achieved through directing the therapy on two targets, the Mϕ and the T cell.

While excessive PGE$_2$-dependent monocytosis, for example, could be counteracted with Indo, as shown in a previous study,[16] we noted in the present investigation that a considerable additive effect can be derived from the combination with TP-5. It appears that TP-5 probably is acting by blocking off some suppressor-active T-cell subpopulation.[27]

The preservation of functionally intact T-cell subpopulations (CD3+, CD4+) also clearly results from synergistic action of both agents, whereas undisturbed IL-2R expression appears to be mainly a function of cyclooxygenase inhibition.[28]

The successful preservation of IL-2 under stressful conditions, evidently, must be the consequence of the smooth interaction of several factors: the presence of adequate numbers of CD4+ cells, the induction of cyclic GMP and simultaneous shut off of cyclic AMP, as well as the presence of IL-1 synthesizing Mϕ, all of which are optimally conditioned via Indo/TP-5 support. Adequate production of γ-IFN from T cells depends on the presence of IL-2 in these cells. Most convincingly in this study, the preoperative serum levels of γ-IFN could only be sustained in those patients (PB) in whom the in vitro stimulation of PBMC cultures also resulted in intact IL-2 synthesis.

In a recent multicenter study with low-dose administration of γ-IFN to patients with multiple trauma, Polk and colleagues[29] showed the mechanistic impact of this mediator for the preservation of Mϕ forward–immunoregulatory capacity in terms of HLA-DR expression after injury.

In a most recent in vivo experimental investigation Ertel et al.[30] demonstrated the potential of maintaining Mϕ function following hemorrhagic shock, with cyclooxygenase inhibition. These investigators showed that the administration of the NSAID ibuprofen resulted in a significant improvement of the antigen presentation capacity, IL-1 and TNF/cachectin synthesis in peritoneal Mϕ.

In a further study using the identical shock model, these authors could find evidence that the administration of ibuprofen following hemorrhage resulted in maintenance of IL-2 and γ-IFN synthesis in splenic lymphocytes as well as of IL-1 in splenic Mϕ from C3H/HeN mice[31] following hemorrhage. In contrast untreated hemorrhaged mice showed massive depression of these respective functions.[31]

The concept of strengthening the facilitory component—while simultaneously suppressing the downregulatory PGE$_2$-mediated component—of Mϕ behavior has also been advocated by Browder and coworkers.[32] In a randomized, prospective, double-blind study they investigated the effect of glucan, a macrophage stimulant, on CMI function in patients with multiple injury and found that glucan treatment resulted in a rapid increase of IL-1 in serum, which was also correlated with subsequent skin test conversion of anergic patients to positive. While the use of such an immunopotentiating agent may represent an intriguing approach to immune response modification, the administration of cyclooxygenase inhibitors, however, is intended to go far beyond stimulation in its therapeutic impact. Nonsteroidal anti-inflamatory drugs, by breaking down the inflammatory cycle, by limiting the nondiscriminant whole-body hyperinflammation, represents in our experience the key step to avoid the overstimulation of the Mϕ during the initial post-traumatic phase. A number of agents, some of them acting in a self-sustaining, autoregulatory fashion, such as PGE$_2$, and IL-6 complement split products or lipopolysaccharides, it seems, cannot turn on the deleterious suppressor mode in the Mϕ after trauma, when the increase of cyclic AMP is counteracted with a NSAID. In this study we could show that the combination of Indo and TP-5 could alleviate the steep increment of the Mϕ metabolite NPT as an indicator of a Mϕ-driven hyperimmune response[24] compared to PC. We postulate that for the intact CMI function, the potentially deleterious acute-phase reaction can be downregulated with Indo/TP-5. This suggestion also has been corroborated by our data (unpublished), which showed a significantly lower IL-6 release in LPS-stimulated PBMC cultures compared to PC.

The analysis of postoperative infectious complications within the patient population studied demonstrated a total of three cases with severe septic episodes with gram-negative bacteria in PA/PC and one case of sternal osteitis through staphylococcus epidermidis. Two of the three patients with sepsis died. In PB there were three cases of minor infectious episodes. Obviously the patient numbers within that specific operative model are too small to allow conclusions in regard to clinical relevance.

This study quantified and specified with a tridimensional set of parameters the massive impact of open heart surgery under ECC on CMI.[33] Scrutiny of the efficacy of

different immunopotentiating substances revealed that combined therapy of Indo and TP-5 is superior to sole Indo administration. Indo/TP-5 therapy resulted in a most adequate protection of all T-cell functions tested and it alleviated postoperative monocytosis and Mϕ overactivation. We found a convincing concurrence between the results of our long-term series of *in vitro* mechanistic studies and the findings derived from the simultaneous *in vivo* administration of blocking, and enhancing, agents in terms of protection of adequate Mϕ/T-cell interaction following major operative trauma.

Acknowledgments

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