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Distinct Mechanisms of Immunosuppression as a Consequence of Major Surgery

THORSTEN HENSLER,[1] HEIKE HECKER,[1] KLAUS HEEG,[2] CLAUS-DIETER HEIDECKE,[1] HOLGER BARTELS,[1] WINFRIED BARTHLEN,[1] HERMANN WAGNER,[2] JÖRG-RÜDIGER SIEWERT,[1] AND BERNHARD HOLZMANN[1]*

Department of Surgery, Klinikum rechts der Isar,[1] and Institute for Medical Microbiology, Immunology, and Hygiene,[2] Technical University, D-81675 Munich, Germany

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Altered host defense mechanisms after major surgery or trauma are considered important for the development of infectious complications and sepsis. In the present study, we demonstrate that major surgery results in a severe defect of T-lymphocyte proliferation and cytokine secretion in response to coligation of the antigen receptor complex and CD28. During the early postoperative course, reduced cytokine secretion was observed for interleukin-2 (IL-2), gamma interferon, and tumor necrosis factor alpha, which are associated with the Th1 phenotype of helper T lymphocytes, and for IL-4, the index cytokine of Th2 cells. During the late postoperative course, T-cell cytokine secretion increased to normal levels. Production of the anti-inflammatory cytokine IL-10 was altered, with different kinetics being selectively elevated during the late postoperative course. In contrast, the capacity of peripheral blood monocytes to present bacterial superantigens and to stimulate T-cell proliferation was normal or enhanced after surgery despite a significant loss of cell surface HLA-DR molecules. Thus, the level of major histocompatibility complex class II protein expression does not appear to predict the antigen-presenting capacity of monocytes obtained from surgical patients with uneventful postoperative recovery. Secretion of IL-1β and IL-10 by endotoxin-stimulated peripheral blood monocytes was increased at different time points after surgery. Major surgery therefore results in a distinct pattern of immune defects with a predominant defect in the T-cell response to T-cell receptor- and CD28 coreceptor-mediated signals rather than an impaired monocyte antigen-presenting capacity. Suppression of T-cell effector functions during the early phase of the postoperative course may define a state of impaired defense against pathogens and increased susceptibility to infection and septic complications.

Impaired host defense mechanisms following major surgery or trauma are considered important for the development of sepsis. Functional alterations of the adaptive immune system have been studied most extensively in patients with severe trauma or burn injury, demonstrating reduced cellular proliferation and secretion of interleukin-2 (IL-2) and gamma interferon (IFN-γ) by mitogen-stimulated T lymphocytes with a concomitant increase in IL-4 production (14–17, 26, 36, 39, 48). However, reduced cell proliferation and IL-2, but not IFN-γ, secretion do not appear to reflect defects intrinsic to T cells but instead may result from inhibitory factors such as prostaglandin E2 that are released from mononuclear phagocytes at elevated levels after injury (14–16). Following major surgery, a reduced delayed-type hypersensitivity response was demonstrated, whereas the mixed-lymphocyte cultures were normal in these patients (37). Failure to respond to recall antigens in skin testing correlated with impaired neutrophil chemotaxis, suggesting altered leukocyte recruitment as an alternative mechanism of suppressed delayed-type hypersensitivity response in these patients (16). In addition, alterations of monocyte functions were reported to include loss of cell surface HLA-DR molecules following trauma or major surgery and reduced secretion of cytokines, including IL-1, IL-6, and IL-8 in patients with major trauma, while a transient increase of monocyte IL-1 production was observed after burn injury (9, 16, 18, 25, 36, 47, 48).

* Corresponding author. Mailing address: Department of Surgery, Klinikum rechts der Isar, Technical University, Ismaninger Str. 22, D-81675 Munich, Germany. Phone: 49-89-4140-2033. Fax: 49-89-4140-4868.

Protective immunity to infection is critically dependent on the regulated production of cytokines and other immune regulators. Targeted disruption of cytokine genes by homologous recombination in mice has established distinct functions of cytokines that are indispensable for the acquired resistance to numerous pathogens. Thus, increased susceptibility to infection with intracellular pathogens such as *Listeria monocytogenes* or mycobacteria was demonstrated in mice lacking tumor necrosis factor alpha (TNF-α) and lymphotoxin-α, the 55-kDa receptor for TNF, IFN-γ, or the receptor for IFN-γ (10, 13, 27, 30, 40, 43). Moreover, humoral immune responses to protein or viral antigens and immunoglobulin isotype switching were severely impaired in mice with disrupted TNF-α, lymphotoxin-α, or IL-4 genes (2, 13, 32). Resistance to vaccinia virus infection and generation of specific cytotoxic T-lymphocyte responses to vaccinia virus or lymphocytic choriomeningitis virus were impaired in mice deficient for TNF-α and lymphotoxin-α or the receptor for IFN-γ (13, 27). While these reports clearly demonstrate selective immunosuppressive effects of cytokine deficiency, the systemic toxicity of cytokines was emphasized by the increased resistance of mice lacking the IFN-γ receptor or the 55-kDa receptor for TNF to shock induced by endotoxin or bacterial superantigens (8, 40, 43).

In the present study, we demonstrate that major surgery results in a severe defect of T cells to proliferate and to secrete cytokines characteristic of both Th1 and Th2 cells, including IFN-γ, IL-2, TNF-α, and IL-4. In contrast, the capacity of monocytes to present antigen was not impaired, although cell surface HLA-DR antigens were downregulated after surgery. Moreover, monocyte secretion of IL-1β and IL-10 was increased at different phases of the postoperative course. The

results therefore suggest that suppression of T-cell functions but not monocyte functions is an important mechanism of immunosuppression observed after major surgery that may contribute to the development of septic complications.

MATERIALS AND METHODS

Patient population and study design. In the study were included 69 patients (41 men and 28 women) with a mean age of 65 ± 9 years. The operations were performed mainly because of malignant disease (83% of patients). Patients were not subjected to neoadjuvant radio- or chemotherapy. At no time did any of the patients develop clinical signs of infectious complications. From each patient, blood samples were collected before the operation as well as 1 to 2 and 3 to 5 days after surgical intervention. For a control, blood samples from 25 healthy individuals were analyzed. Informed consent was obtained from patients in all cases, and the study received local hospital ethical committee approval.

Cell culture and antibodies. Murine monoclonal antibodies used in this study were directed against CD3-ε (X35), CD14 (RM052), CD28 (CD28.2), T-cell receptor (TCR) αβ (BMA031), HLA-DR (B8.12.2), or L-selectin (DREG56). Antibodies and isotype-matched control immunoglobulins directly conjugated with fluorescein isothiocyanate or *R*-phycoerythrin were purchased from Immunotech, Hamburg, Germany. For three-color flow cytometry analysis, CD4 antibody FK3 conjugated with PerCP was obtained from Becton Dickinson (Heidelberg, Germany). Cell cultures were performed in RPMI 1640 medium containing 7% heat-inactivated fetal calf serum, 100 μg of streptomycin per ml, and 100 U of penicillin per ml (Biochrom, Berlin, Germany).

Isolation of peripheral blood T cells and monocytes. Human peripheral blood mononuclear cells (PBMCs) were isolated from 25 ml of heparinized blood by Ficoll-metrizoate density gradient centrifugation. Isolated PBMCs were washed twice with phosphate-buffered saline (PBS), and the total cell count was determined. PBMCs were plated in six-well tissue culture plates (Nunc, Roskilde, Denmark) in a total of 2 ml of PBS and incubated for 1 h at 37°C. Adherent cells were removed from culture dishes with disposable cell scrapers (Nunc). Adherence-purified monocytes were washed and resuspended in RPMI 1640 cell culture medium. Nonadherent cells were collected, washed with PBS, and resuspended in RPMI 1640 cell culture medium. T cells were enriched by depletion of B cells and monocytes from nonadherent cells with immunomagnetic beads coated with CD14 and CD19 antibodies according to the manufacturer's instructions (Dynal, Oslo, Norway).

Cytokine secretion of peripheral blood T cells and monocytes. Enriched T cells were placed into 24-well tissue culture plates (Nunc) that had been precoated for 1 h at 37°C with 250 μl of a 20-μg/ml solution of CD3 antibody. CD28 antibody and goat anti-mouse immunoglobulin were added to T cells at 5 μg/ml each, and T lymphocytes were stimulated for 16 h. Cytokine production of monocytes purified by plastic adherence was stimulated by incubation with 1 μg of endotoxin per ml from *Escherichia coli* serotype O127:B8 (Sigma Chemical Co., St. Louis, Mo.) for 16 h. After stimulation, all supernatants were centrifuged to remove residual cells and stored at −20°C until analysis.

The levels of IL-1β, IL-2, IL-4, IL-10, IFN-γ, and TNF-α (Medgenix Diagnostics, Fleurus, Belgium) as well as IL-12 (Biermann, Bad Nauheim, Germany) in supernatants of stimulated T cells or monocytes were determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. All cytokine assays were standardized by inclusion of a titration of the appropriate purified recombinant cytokine of known concentration. The levels of sensitivity of the ELISAs were 2 pg/ml (IL-1β), 0.1 IU/ml (IL-2), 2 pg/ml (IL-4), 1 pg/ml (IL-10), 5 pg/ml (IL-12), 0.03 IU/ml (IFN-γ), and 3 pg/ml (TNF-α). The absorbance of the samples was determined on an MRX Microplate Reader (Dynatech, Denkendorf, Germany) with 450 nm as the primary wavelength and 630 nm as the reference wavelength.

Proliferative response of peripheral blood T cells. To determine the proliferative response of circulating T cells, two distinct protocols were applied. In one set of experiments, T-cell proliferation was stimulated by cross-linking of CD3 and CD28 receptors. Round-bottom 96-well tissue culture plates (Nunc) were coated with CD3 antibody by incubation of 50 μl of a 20-μg/ml solution for 1 h at 37°C. To each well, 2×10^4 enriched T cells were added together with CD28 antibody and goat anti-mouse immunoglobulin (5 μg/ml each). Alternatively, T-cell proliferation was induced by addition of 20 ng of each bacterial superantigen per ml to unfractionated PBMCs (2×10^4/well). The concentrations of superantigens used were found to result in maximal stimulation of T-cell proliferation. T cells were stimulated for 5 days, and DNA synthesis was measured by addition of 18.5 kBq of [^{3}H]thymidine per well (Amersham Buchler, Braunschweig, Germany) 24 h before termination of the experiment. Time course experiments revealed that under the experimental conditions used, plateau levels of [^{3}H]thymidine incorporation were reached after 5 to 7 days of stimulation. The samples from triplicate cultures were collected onto glass fiber filters (Bibby Dunn, Asbach, Germany), and radioactivity was determined with a Matrix 96 Beta Counter (Packard Instruments, Frankfurt, Germany).

Immunofluorescence staining and flow cytometry analysis. Expression of cell surface molecules on circulating T cells and monocytes was analyzed by two- and three-color immunofluorescence staining. Saturating concentrations of monoclonal antibodies were incubated with 100 μl of heparinized blood. For a control, isotype-matched mouse immunoglobulins were included in each experiment. Erythrocytes were lysed by addition of 10 volumes of fluorescence-activated cell sorter lysing solution (Becton Dickinson). Cells were washed with PBS and fixed in PBS containing 2% paraformaldehyde. Fluorescence was analyzed on an EPICS XL cytometer (Coulter Corporation, Hialeah, Fla.). Instrument calibration was performed daily with Calibrite Beads (Becton Dickinson) according to the recommendations of the manufacturer. T cells and monocytes were identified by forward and side scatter profile and positive staining with antibodies to the TCR αβ heterodimer or CD14, respectively.

Statistical analysis. Statistical analysis of data obtained from consecutive blood samples was performed with the Mann-Whitney U test for paired samples. To compare the results from healthy volunteers with those from surgical patients (preoperative values), the Mann-Whitney U test for unpaired samples was used. Results are presented as mean values ± standard errors (SE). The level of significance was set at $P < 0.05$.

TABLE 1. Clinical profile of patients undergoing major elective surgery in this study[a]

Type of surgical procedure	No. of patients
Esophagectomy	18
Total gastrectomy	12
Partial pancreatoduodenectomy	14
Left hemicolectomy	6
Radical sigmoid resection	4
Anterior resection of the rectum	7
Miscellaneous resectional surgery	8
Total	69

[a] In 83% of the patients, surgery was performed because of malignant disease. Patients were not subjected to neoadjuvant radio- or chemotherapy and did not develop clinical signs of infectious complications.

RESULTS

Altered cytokine response of peripheral T cells is associated with major surgery. It has been proposed that major surgery or trauma may induce a state of immunosuppression that increases the risk for the development of sepsis. To identify altered immune functions associated with major surgery, analysis of peripheral blood T lymphocytes was performed before surgery and during the early (days 1 and 2) and late (days 3 to 5) phases of the postoperative course. In the present study, 69 patients showing uneventful recovery have been included. The clinical profiles of these patients are detailed in Table 1. In the majority of patients, surgery was performed because of malignant disease.

T-cell-enriched fractions of PBMCs from these patients were obtained by removal of B cells and monocytes, and cytokine production was stimulated by antibody-mediated cross-linking of CD3 and CD28 molecules. The results in Fig. 1 clearly demonstrate that the production of cytokines associated with the Th1 phenotype of CD4 T cells was strongly impaired following major surgery. When compared to the levels of T-cell cytokine production before operation, secretion of cytokines during the early phase of the postoperative course was reduced by 52% for IFN-γ, 55% for IL-2, and 44% for TNF-α. During the late postoperative course, production of IL-2 and IFN-γ increased, reaching levels similar to those observed before surgery (Fig. 1). In contrast, TNF-α production of T cells remained suppressed for a prolonged period of time, with a significant reduction also observed during the late postoperative course (Fig. 1). The results of stimulated cytokine production of T cells from representative patients are presented in Table 2, confirming that the cytokine secretion profile of T cells is altered after surgery.

To control for spontaneous cytokine production by T cells or contaminating leukocytes, TNF-α, which may be secreted by

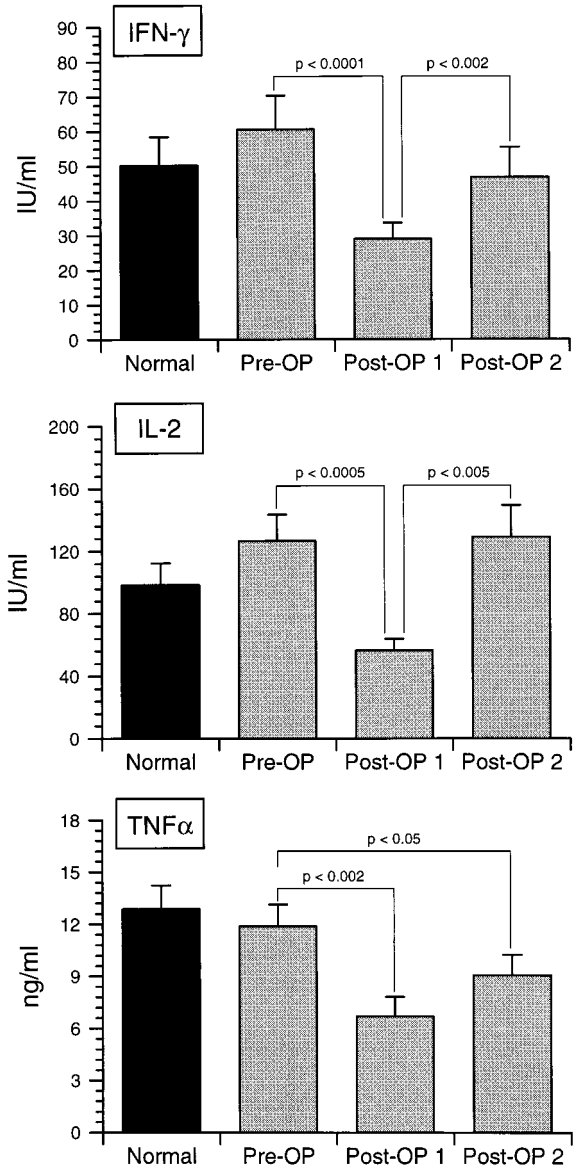

FIG. 1. Secretion of Th1-type cytokines by activated T cells is impaired after surgery. Blood samples were collected on consecutive days for each patient or from healthy volunteers, and T cells were enriched from nonadherent PBMCs by depletion of B cells and monocytes with immunomagnetic beads coated with CD19 and CD14 monoclonal antibodies. T cells were stimulated by antibody-mediated cross-linking of CD3 and CD28 for 16 h, and supernatants were analyzed for cytokine secretion by specific ELISA. Cytokine production of 10^5 T cells is given, and results are presented as means ± SE. Pre-OP, preoperation; Post-OP1 and 2, 1 to 2 and 3 to 5 days postoperation, respectively.

multiple cell types, was measured from unstimulated cultures. The results showed that TNF-α secretion increased by about 20-fold after stimulation with CD3 and CD28. The values were 0.50 ± 0.19 ng/ml before surgery, 0.43 ± 0.12 ng/ml for the early postoperative phase, and 0.66 ± 0.19 ng/ml for the late postoperative course. These data therefore suggest that cytokine levels determined after CD3 and CD28 stimulation of T cells do not reflect spontaneous cytokine release by contaminating leukocytes or preactivated T cells.

Secretion of IL-4, which is associated with the Th2 phenotype of CD4 T cells, was also affected by major surgery. The data presented in Fig. 2 and Table 2 (representative patients) demonstrate that production of IL-4 was reduced by 26% during the early phase of the postoperative course, but returned to levels similar to those observed during the late postoperative course. In contrast, IL-10 secretion was not suppressed but instead was significantly increased late after surgery. Collectively, these results indicate that major surgery is associated with a severe, but transient reduction in the capacity of T cells to secrete a large panel of cytokines with the notable exception of IL-10.

Effect of major surgery on the proliferative response of peripheral T cells. To further define the effects of major surgery on T-cell function, the proliferative response after ligation of CD3 and CD28 receptors was examined. For analysis of T-cell proliferation, PBMCs were depleted of monocytes and B cells to exclude the possibility that functional alterations of a patient's antigen-presenting cells (APCs) may affect the results. As shown in Fig. 3, proliferation of T cells was reduced by 30% during the early phase of the postoperative course. The reduction of the T-cell proliferative response was transient, however, and during the late postoperative course, CD3/CD28-stimulated proliferation returned to values not different from those observed before surgery (Fig. 3). Thus, major surgery is also associated with a reduced proliferative capacity of peripheral T cells.

Cell surface phenotype of circulating T cells and monocytes after major surgery. It has been well established that major surgery or trauma is associated with a loss of cell surface HLA-DR expression on circulating monocytes (9, 25, 47). The results depicted in Fig. 4 confirm these findings and demonstrate that during the early postoperative course of patients undergoing major surgery, the mean fluorescence intensity of HLA-DR expression on CD14-defined circulating monocytes was decreased by 61% compared to preoperative values. Moreover, monocyte expression of HLA-DR remained suppressed by a similar extent during the late postoperative course (Fig. 4A). Immunofluorescence analysis revealed that changes in HLA-DR expression were characterized as a shift in intensity of a single peak of fluorescence rather than by the appearance of distinct subpopulations, suggesting that loss of cell surface expression on peripheral monocytes does not result from selective depletion of subsets expressing high levels of HLA-DR.

On T lymphocytes, expression of HLA-DR molecules is associated with cell activation. The results of three-color immunofluorescence analyses demonstrated a transient and significant increase in the fraction of $CD4^+$ T cells expressing HLA-DR during the early postoperative course (Fig. 4B). In addition, expression of HLA-DR on $CD4^+$ T cells was constitutively elevated in surgical patients compared to that of healthy controls (Fig. 4B). These results therefore indicate that an increased number of activated $CD4^+$ T cells are present in the circulation early after surgery.

Differential migration of subsets of circulating cells may affect analysis of T-lymphocyte and monocyte functions. To address this question, cell surface adhesion molecule expression was examined. The results presented in Fig. 4 show that the number of $CD4^+$ T cells and monocytes expressing L-selectin did not change significantly during the entire observation period. Similarly, expression of CD11b on circulating monocytes was not altered after surgery (data not shown). However, when compared with that in healthy volunteers, the number of L-selectin-positive $CD4^+$ T cells and monocytes was reduced in surgical patients. In summary, analysis of expression of cell surface adhesion molecules does not provide evidence for an altered migratory capacity of circulating cells.

Normal antigen-presenting capacity of circulating monocytes after major surgery. To analyze whether the loss of

TABLE 2. CD3-plus-CD28-stimulated cytokine secretion of T lymphocytes from individual patients[a]

Patient	Cytokine secretion														
	IFN-γ (IU/ml)			IL-2 (IU/ml)			TNF-α (ng/ml)			IL-4 (pg/ml)			IL-10 (pg/ml)		
	Pre-OP	Post-OP 1	Post-OP 2	Pre-OP	Post-OP 1	Post-OP 2	Pre-OP	Post-OP 1	Post-OP 2	Pre-OP	Post-OP 1	Post-OP 2	Pre-OP	Post-OP 1	Post-OP 2
1	14.8	2.0	6.8	101	46	73	9.9	0.1	3.9	560	403	743	124	82	245
2	5.5	3.9	2.6	260	64	334	7.5	0.8	13.9	569	448	480	124	112	132
3	46.4	26.1	58.1	193	137	231	9.5	4.6	0.5	412	513	733	1,084	1,443	477
4	20.7	7.1	39.9	205	69	367	9.4	2.6	15.7	1,376	650	807	259	61	241
5	37.1	20.1	17.9	129	51	93	21.3	3.4	5.1	205	141	367	219	134	90
6	26.4	11.5	35.5	100	18	35	15.2	2.8	9.4	123	58	216	108	209	87
7	44.7	12.8	14.6	24	24	25	1.1	1.4	1.9	67	70	51	505	223	764
8[b]	58.5	20.1	23.4	463	55	206	24.8	18.6	11.9	251	92	163	251	200	199
9	88.8	17.6	44.9	454	12	222	23.5	4.0	19.4	467	99	207	297	295	1,045
10	61.6	37.0	96.8	65	28	522	11.0	4.5	24.8	41	32	429	405	189	364
11	219.4	168.4	92.8	355	171	119	24.0	21.7	13.5	321	215	151	293	767	378
12	45.9	18.4	43.3	226	10	623	16.6	1.0	22.6	263	40	509	547	645	484
13[b]	96.4	19.9	37.5	110	4	127	9.9	0.1	10.3	78	43	29	571	298	1,934
14	253.1	9.5	76.2	192	8	22	2.3	0.6	4.1	70	18	63	1,647	303	3,451
15	39.2	27.3	55.8	44	32	265	9.4	2.0	16.5	97	42	158	301	262	421

[a] Cytokine secretion profiles of T cells from individual patients are shown. Cytokine production was examined as described in the legends to Fig. 1 and 2. Pre-OP, preoperation; Post-OP 1 and 2, 1 to 2 and 3 to 5 days postoperation, respectively.
[b] Patients with nonmalignant disease.

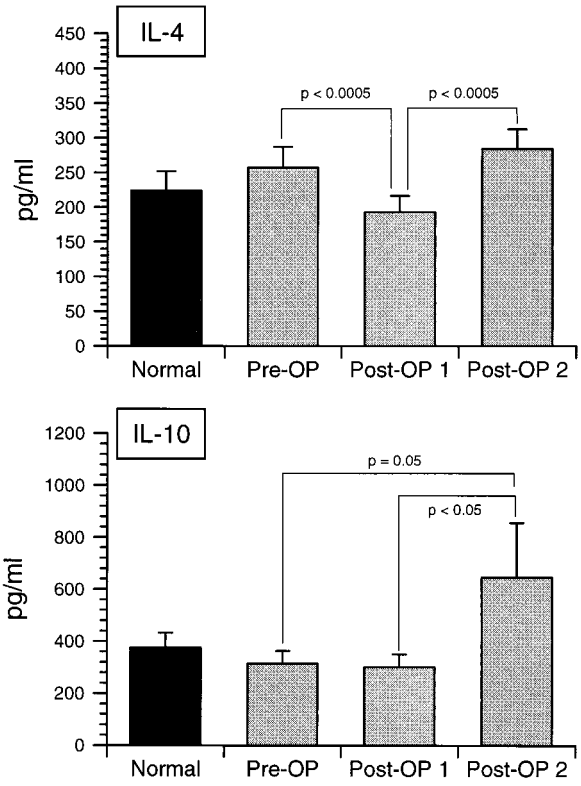

FIG. 2. Differential effect of major surgery on IL-4 and IL-10 production by activated T lymphocytes. T cells were enriched from nonadherent PBMCs isolated from consecutive blood samples of each patient or from healthy volunteers by depletion of B cells and monocytes with CD19 and CD14 immunomagnetic beads and were stimulated by cross-linking of CD3 and CD28 for 16 h. Supernatants were analyzed for secretion of IL-4 and IL-10 by specific ELISA. Cytokine production of 10^5 T cells is given, and results are presented as means ± SE. Pre-OP, preoperation; Post-OP 1 and 2, 1 to 2 and 3 to 5 days postoperation, respectively.

HLA-DR cell surface expression may affect the antigen-presenting capacity of peripheral blood monocytes, unfractionated PBMCs were incubated with the bacterial superantigens staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB), and toxic shock syndrome toxin 1 (TSST-1), and APC-dependent T-cell proliferation was determined. As depicted in Fig. 5, superantigens presented by endogenous monocytes strongly stimulated the proliferative response of T cells during the entire postoperative course. Interestingly, T-cell proliferation was not suppressed after surgery compared to the response before the operation (Fig. 5). Upon stimulation with TSST-1, even enhanced T-cell proliferation was observed, with

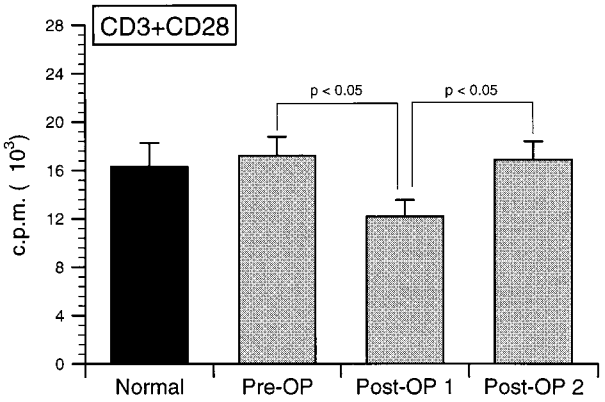

FIG. 3. Reduced APC-independent T-cell proliferation following major surgery. Blood samples were collected on consecutive days for each patient or from healthy volunteers, and T cells were enriched from nonadherent PBMCs by depletion of B cells and monocytes with CD19 and CD14 immunomagnetic beads. T cells were stimulated by cross-linking of CD3 and CD28 for 5 days, and proliferation was determined by incorporation of [^{3}H]thymidine. The proliferative response of 2×10^4 T cells is given. Values for spontaneous incorporation of [^{3}H]thymidine in the absence of antibodies have been subtracted and were 48.5 ± 9.4 cpm (preoperation [Pre-OP]), 77.7 ± 36.2 cpm (1 to 2 days postoperation [Post-OP 1]), and 64.6 ± 19.6 cpm (3 to 5 days postoperation [Post-OP 2]). Incorporation of cells stimulated by ligation with anti-CD28 alone was not significantly different from that of medium controls (data not shown). All results are presented as means ± SE.

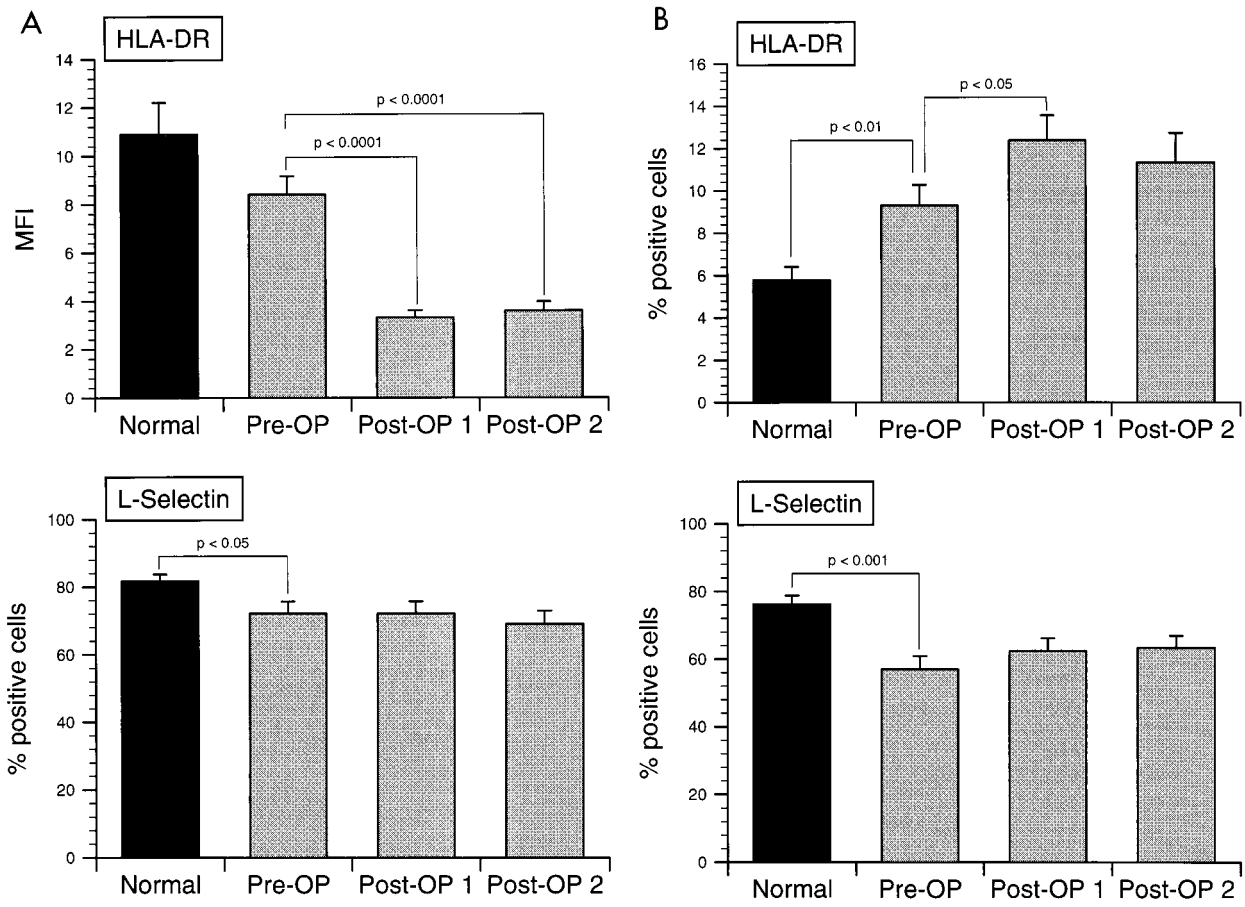

FIG. 4. Cell surface phenotype of circulating monocytes and T cells after major surgery. (A) Expression of HLA-DR and L-selectin on circulating monocytes was analyzed by two-color flow cytometry. Monocytes were identified by forward and side scatter profiles and expression of CD14. Expression of HLA-DR was recorded as mean fluorescence intensity, whereas for L-selectin, the percentage of positive cells is given. Values obtained with isotype-matched control immunoglobulin have been subtracted. Results are presented as means ± SE. (B) Expression of HLA-DR and L-selectin on circulating T cells was analyzed by three-color flow cytometry. T cells were identified by forward and side scatter profiles and expression of the TCR αβ heterodimer and gated for the $CD4^+$ subset. Percentages of positive cells corrected for values obtained with isotype-matched control immunoglobulin are given. Results are presented as means ± SE. Pre-OP, preoperation; Post-OP1 and 2, 1 to 2 and 3 to 5 days postoperation, respectively. MFI, mean fluorescence intensity.

an increase of 28% during the early phase of the postoperative course (Fig. 5). These results therefore indicate that the capacity of circulating monocytes to present major histocompatibility complex (MHC) class II-dependent antigens, such as bacterial superantigens, to T cells and to stimulate proliferation is not suppressed after major surgery, despite a significant loss of cell surface HLA-DR molecules.

Effect of major surgery on monocyte cytokine production. In additional experiments, the effect of major surgery on cytokine production by peripheral blood monocytes was examined. Monocytes were purified from PBMCs by plastic adherence, and cytokine secretion was stimulated by incubation with endotoxin. Interestingly, the results presented in Fig. 6 reveal that monocyte secretion of IL-1β was elevated by about twofold during the early phase of the postoperative course, whereas late after surgery, IL-1β production decreased to preoperative levels. Alterations of IL-10 production by endotoxin-stimulated monocytes revealed different kinetics with a significant increase observed during the late postoperative course (Fig. 6). In contrast, monocyte IL-12 production was not altered after surgery. Secretion of TNF-α appeared increased during the late postoperative course, but differences from preoperative levels did not reach statistical significance (Fig. 6). Together, the results therefore indicate that major surgery is associated with an increased secretion of cytokines by peripheral blood monocytes that selectively affects IL-1β and IL-10 at different time points after an operation.

DISCUSSION

Activation of naive T cells requires costimulatory signals provided by soluble factors or interaction of cell surface-bound receptors that act in conjunction with TCR-mediated events (5, 6). Ligation of CD28 by antibodies or natural ligand has been shown to provide potent cosignals that promote cell cycle progression of T cells and increased IL-2 production (reviewed in references 4, 28, 29, 34, 35, and 46). Analysis of gene-deficient mice directly demonstrated the importance of CD28-mediated costimulatory signals for T helper cell-dependent antibody production and induction of CD8 T-cell responses after short-term exposure to viral antigen (33, 45). Moreover, in vitro mitogen stimulation revealed diminished proliferation and IL-2 production of T cells derived from CD28-deficient mice (45). In the present study, T cells from patients subjected to major surgery were activated by antibody-mediated ligation of the TCR-CD3 complex and the CD28 receptor in the absence

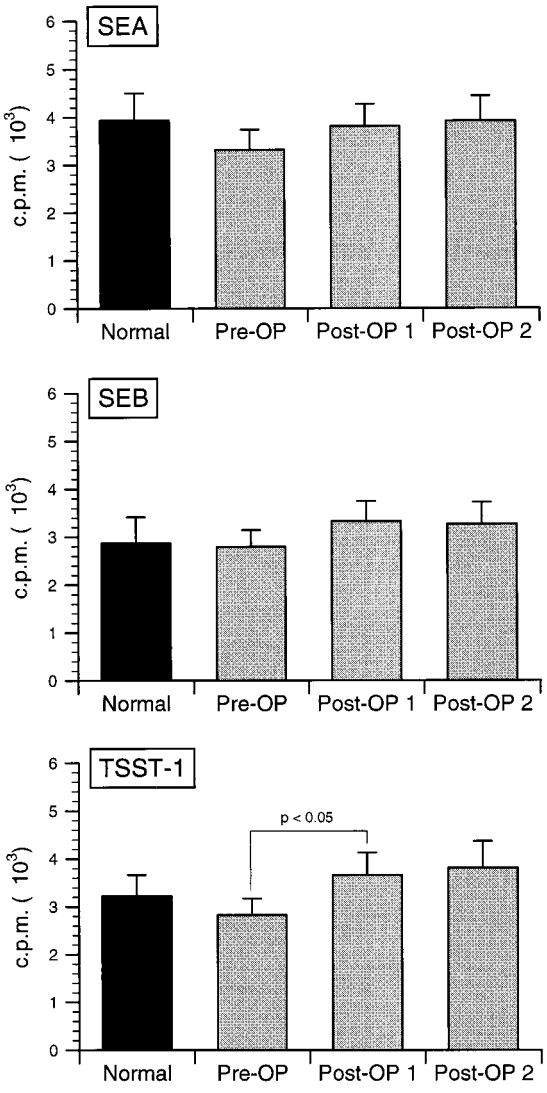

FIG. 5. T-cell proliferation stimulated by superantigens and the patient's APCs is not impaired following surgery. PBMCs derived from consecutive blood samples of patients or from healthy volunteers were stimulated by incubation with optimal concentrations of the bacterial superantigen SEA, SEB, or TSST-1 for 5 days. The proliferative response obtained from 2×10^4 PBMCs was determined by [^{3}H]thymidine incorporation. Values for spontaneous proliferation of T cells in the absence of bacterial superantigens have been subtracted and were 169.8 ± 36.2 cpm (preoperation [pre-OP]), 186.6 ± 45.6 cpm (1 to 2 days postoperation [post-OP 1]), and 181.1 ± 85.6 cpm (3 to 5 days postoperation [post-OP 2]). All results are presented as means ± SE.

of APCs. We demonstrate that stimulation of peripheral T cells is severely impaired during the early phase of the postoperative course, resulting in both reduced cytokine secretion and cell proliferation. Thus, defective signaling through the TCR-CD3 complex and CD28 may represent an important mechanism of immunosuppression following major surgery, resulting in impaired host defense and increased susceptibility to infection and sepsis.

CD4 T lymphocytes may differentiate into functionally distinct T helper cell populations that are characterized by different patterns of cytokine secretion (38, 42, 44). Th1 cells secrete IFN-γ, IL-2, and lymphotoxin-α and induce both humoral and cell-mediated immune responses, while Th2 cells produce IL-4, IL-5, IL-6, and IL-13, providing help for humoral immune responses. Numerous studies have demonstrated that unbalanced differentiation of Th1 and Th2 cells during an immune response may result in severely impaired defense against diverse pathogens (42). Interestingly, functional analysis of T lymphocytes obtained from patients after major surgery revealed a substantial defect in cytokine secretion that involved both Th1- and Th2-type cytokines, including IFN-γ, IL-2, and IL-4. In contrast, production of IL-10 was not suppressed during the early phase of the postoperative course and was even increased late after surgery. Although IL-10 synthesis is associated with the Th2 phenotype of murine T helper cells, evidence was provided suggesting that human IL-10 is also secreted by Th1 cells (11). It therefore appears that major surgery is associated with a severe defect of T-cell cytokine secretion that is characterized by the predominant production of the anti-inflammatory cytokine IL-10 rather than a polarized differentiation to a Th1 or Th2 phenotype.

Major trauma and burn injury have been reported to result in reduced proliferation and secretion of IL-2 and IFN-γ by mitogen-stimulated T lymphocytes (14–17, 26, 36, 39, 48). However, unlike our observations showing a transient defect of T-cell functions 1 to 2 days after major surgery, impaired T-cell responses as a consequence of thermal or mechanical trauma were found to persist for a long period of time after injury. For example, in patients with burns over greater than 30% of the body surface area, reduced IL-2 production was demonstrated for up to 60 days after injury (48). Moreover, recent studies suggest that serious injury may induce polarization of T helper cells to the Th2 as opposed to the Th1 phenotype rather than causing generalized T-cell suppression. This conclusion was based on the finding that T cells from burn and trauma patients produce less IFN-γ than control cells, whereas secretion of IL-4, the index cytokine of Th2 cells, was increased (39). Augmented IL-4 production after thermal or mechanical injury therefore contrasts with reduced T-cell secretion of IL-4 after major surgery (Fig. 2). Thus, the results presented here reveal that trauma and major surgery affect T-cell function with distinct kinetics and indicate fundamental differences in the mechanisms that alter T-cell cytokine secretion.

In human peripheral blood T cells, the NF-κB/rel family proteins c-Rel, p50, and RelA are associated with the CD28-responsive element present in the promoter of the IL-2 gene in response to ligation of CD3 and CD28 (20). Moreover, c-Rel cotransfection augments expression of reporter genes linked to the CD28-responsive element (20). Consistent with these findings, T lymphocytes from c-Rel-deficient mice are largely unresponsive to a number of mitogenic stimuli, and IL-2 production is reduced in activated T cells (31). Interestingly, CD3- and CD28-stimulated production of numerous other cytokines, including TNF-α, IFN-γ, IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor, is also severely suppressed in c-Rel-deficient T cells (19). The phenotype of c-Rel-deficient T cells is therefore reminiscent of the functional defects observed in T cells of patients following major surgery. Thus, it is tempting to speculate that immune defects associated with major surgery might also involve impaired function of the NF-κB/rel family protein c-Rel.

Consistent with previous results, cell surface levels of HLA-DR antigens were found to be severely suppressed on circulating monocytes of patients following major surgery (9, 25, 36, 47). Loss of cell surface HLA-DR was suggested to reduce the antigen-presenting capacity of monocytes, resulting in impaired T-cell stimulation. To address this question in more detail, we have stimulated PBMCs with a panel of bacterial superantigens including SEA, SEB, and TSST-1. In contrast to conventional protein antigens, superantigens of bacte-

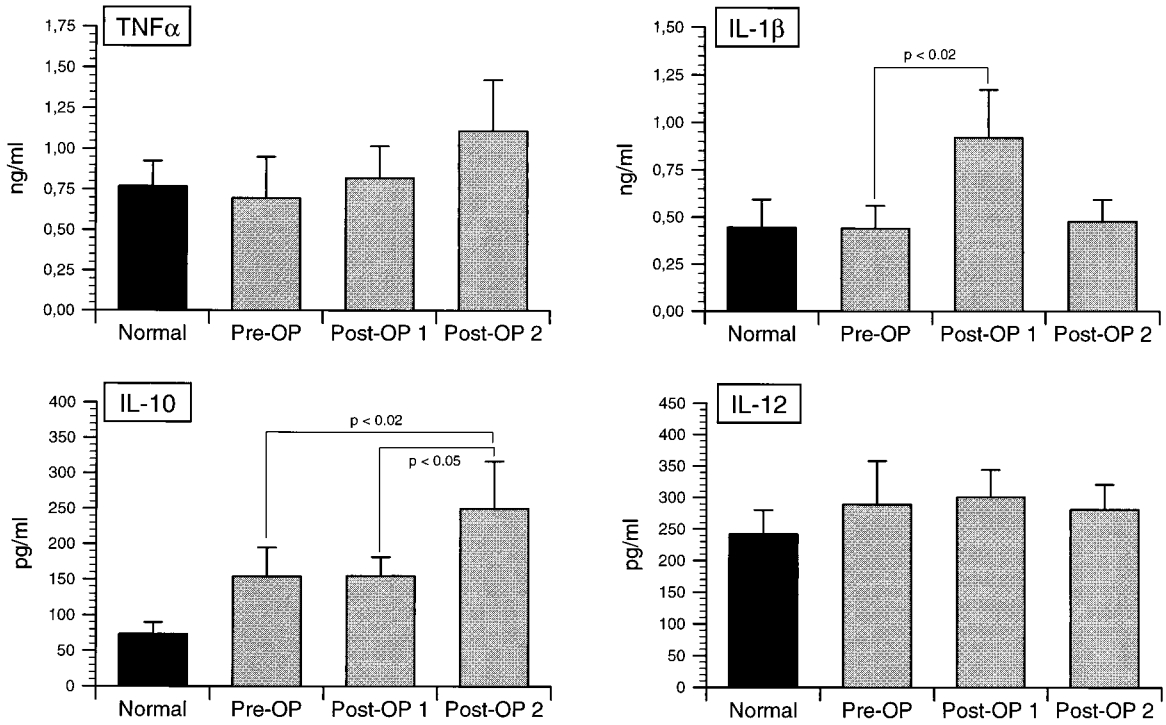

FIG. 6. Elevated production of IL-1β and IL-10 by endotoxin-stimulated monocytes at different time points after major surgery. Monocytes were purified from PBMCs of consecutive blood samples from patients or from healthy volunteers by plastic adherence and stimulated with 1 μg of endotoxin per ml for 16 h. Supernatants were collected, and cytokine secretion was determined by specific ELISA. Results represent cytokine production of 10^5 adherence-purified monocytes and are presented as means ± SE. Pre-OP, preoperation; Post-OP 1 and 2, 1 to 2 and 3 to 5 days postoperation, respectively.

rial and viral origin are bound to conserved regions of the TCR β subunit, which are encoded by specific Vβ gene segments. Therefore, T-cell recognition of superantigens is independent of the clonal specificity of the TCR and results in a multiclonal yet TCR-dependent and Vβ-selective response (for review, see references 1 and 22 to 24). Depending on the Vβ specificity of a given superantigen and the number of individual Vβ segments per genome, superantigens may interact with as many as 2 to 5% of peripheral T cells. In addition, superantigens do not require endocytosis and proteolytic processing by APCs for presentation to T cells but interact with MHC class II molecules as intact proteins at a site distinct from the peptide binding groove. Thus, bacterial superantigens allow for dissection of the complex process of antigen presentation and restrict analysis to distant events such as loading of MHC class II molecules and T-cell costimulation.

Interestingly, activation of T lymphocytes with bacterial superantigens that were presented by the patient's APCs was not impaired after major surgery despite a significant loss of HLA-DR on circulating monocytes and an intrinsic defect of T cells to proliferate in response to ligation of CD3 and CD28 during the early phase of the postoperative course. The question therefore arises of whether the mere number of MHC class II molecules determines the antigen-presenting capacity of monocytes. In fact, previous studies have demonstrated that the efficiency of presentation of the bacterial superantigen SEA and stimulation of T cells is not related to different levels of MHC class II molecules expressed by various types of human APCs but rather reflects the ability of these cells to transmit costimulatory signals (3). Moreover, occupancy of as few as 0.1% of all MHC class II molecules present on dendritic cells (corresponding to an average of 2,000 binding sites per cell) was found to be sufficient for stimulation of quiescent T cells (3). Consistent with these results, quantification of MHC class II-peptide complexes on murine APCs revealed that T cells were activated when only a few hundred or less than 0.1% of all cell surface MHC class II molecules were loaded with specific peptide (12, 21). Thus, with an estimated number of about 10,000 high-affinity binding sites for SEA expressed on human peripheral blood monocytes (3), the number of antigen-presenting complexes may be sufficient to induce superantigen-dependent T-cell proliferation despite the loss of about 60% of HLA-DR molecules following major surgery. In addition, enhanced secretion of IL-1β that was demonstrated during the early phase of the postoperative course may improve the APC function of monocytes. Together, these data therefore suggest that the capacity of circulating monocytes to present antigen and to stimulate proliferation of T cells is not suppressed during uneventful recovery after major surgery, despite a significant loss of cell surface HLA-DR proteins.

Previous studies demonstrated a transient increase of IL-1 production after burn injury, whereas major trauma was shown to result in suppression of IL-1, IL-6, and IL-8 secretion, suggesting that monocyte cytokine secretion is differentially affected by mechanical and thermal injury (16, 48). The present study extends the functional analysis of monocytes to patients undergoing major surgery. The results clearly demonstrate that IL-1β secretion was transiently elevated during the early phase of the postoperative course, while IL-10 secretion was increased late after surgery. In contrast, production of IL-12 and TNF-α was not altered significantly. The results therefore indicate that in contrast to T lymphocytes, monocyte cytokine secretion was not suppressed after surgery. In addition, these findings further support the conclusion that major surgery and

TABLE 3. Proliferative response of T lymphocytes from individual patients[a]

Patient	No. of T lymphocytes proliferating											
	CD3 + CD28			SEA			SEB			TSST-1		
	Pre-OP	Post-OP 1	Post-OP 2	Pre-OP	Post-OP 1	Post-OP 2	Pre-OP	Post-OP 1	Post-OP 2	Pre-OP	Post-OP 1	Post-OP 2
1	24,988	90	13,420	5,258	5,323	12,911	5,768	2,772	12,316	2,705	1,797	11,019
2	20,178	11,331	26,196	6,920	12,957	4,193	5,791	8,559	5,366	6,492	8,817	5,279
3	29,592	1,494	3,930	3,576	7,000	5,583	3,124	6,674	4,017	2,681	6,255	5,105
4	27,380	3,102	32,070	3,252	10,392	17,155	4,474	10,774	15,406	6,862	14,929	18,210
5	15,028	7,833	32,995	876	5,882	854	798	6,130	968	534	5,376	1,161
6	27,778	58	21,736	4,436	163	3,853	1,410	46	2,613	5,996	27	3,003
7	14,636	2,275	22,555	3,130	4,843	3,870	3,825	2,189	2,477	3,907	4,330	3,973
8[b]	25,716	20,707	26,948	1,400	2,139	10,511	3,096	4,534	9,220	1,660	3,991	9,403
9	25,741	413	29,901	511	746	2,045	543	981	3,223	577	1,441	3,411
10	26,167	19,264	26,153	1,001	3,486	3,974	689	1,667	3,857	827	2,376	3,525
11	26,480	21,005	36,724	1,732	1,287	2,545	247	753	1,171	741	1,109	1,112
12	35,094	4,453	25,169	4,390	442	5,109	3,698	369	4,039	2,189	461	5,462
14	14,447	15,950	4,229	6,166	7,883	1,102	1,590	7,170	593	3,284	10,669	1,275
15	28,922	30,375	32,950	1,771	3,362	21,553	1,684	3,313	16,247	2,204	3,894	24,025

[a] Proliferative responses of T cells from individual patients are shown. Proliferation was stimulated by CD3 and CD28 ligation on enriched T cells or incubation of PBMCs with bacterial superantigens as described in the legends to Fig. 3 and 5. Pre-OP, preoperation; Post-OP 1 and 2, 1 to 2 and 3 to 5 days postoperation, respectively.

[b] Patient with nonmalignant disease.

trauma cause distinct functional alterations of the immune system.

In contrast to naive T cells that recirculate almost exclusively through secondary lymphatic organs, memory cells and immunoblasts can also access extralymphoid immune effector sites (7). Thus, it is conceivable that altered migration of memory and activated effector cells may influence the results of cell proliferation and cytokine secretion analysis. Memory and activated or effector T lymphocytes are characterized by increased expression of activation markers such as MHC class II molecules and are enriched for cells lacking L-selectin (41). Consistent with a previous report (47), we demonstrate that the number of $CD4^+$ T cells expressing HLA-DR is transiently increased during the early postoperative phase. Moreover, expression of L-selectin on $CD4^+$ T cells and monocytes did not change during the entire observation period. Similar results have been obtained for CD11b expression on circulating monocytes. Moreover, changes in monocyte HLA-DR expression were characterized in all cases as a shift of a single peak of fluorescence, refuting the possibility that a subpopulation of $HLA\text{-}DR^{high}$ cells may be sequestered at the site of surgery. Together, these results indicate that altered lymphocyte and monocyte functions after surgery do not result from selective depletion of functional subsets from the circulating pool.

The majority of patients analyzed in the present study underwent surgery because of malignant disease. However, the results of cytokine secretion and cell proliferation assays revealed that before surgery, T-lymphocyte and monocyte functions of patients with malignancy are not significantly different from those of healthy volunteers. Data from individual patients with nonmalignant disease are presented in Tables 2 and 3, indicating functional alterations of T cells similar to those observed in patients with malignancy. These data suggest that constitutive levels of immune functions are not compromised by malignancies. However, the findings presented in this study do not exclude the possibility that immune defects related to malignant disease may only become detectable in response to surgical stress. In addition, due to the age profile of the study group, we cannot rule out age as a factor contributing to the immunosuppression following major surgery.

In conclusion, the results of the present study identify suppression of T-cell function rather than monocyte function as a critical mechanism of immunosuppression following major surgery. Together with previous studies, our findings also suggest that despite certain similarities, alterations of the immune system resulting from major surgery significantly differ from those observed after severe thermal or mechanical injury. Characterization of molecular mechanisms of immunosuppression that are associated with major surgery may help to identify patients with an increased susceptibility to infectious complications and to develop novel concepts for postoperative immunomodulation.

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