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ORIGINAL ARTICLE

Immunosuppressive effects of surgery assessed by flow cytometry in nonhuman primates after nephrectomy

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Summary

Despite previous studies suggesting that surgery cause immune suppression, the underlying biologic mechanisms have not been studied using advanced immune function assays. Unilateral nephrectomy was performed in nonhuman primates. Blood was collected before surgery and at different time-points through 14 days after surgery. Lymphocyte proliferation (expression of proliferating cell nuclear antigen in cells in S/G_2M-phase), production of intracellular cytokines [interleukin (IL)-2, interferon (IFN)-γ, tumor necrosis factor (TNF)-α] and expression of surface-activation antigens (CD25, CD71) on T-lymphocytes were assessed in whole blood using flow cytometry. Results were compared with nonoperated control animals. The procedure caused a decrease of 25% in absolute lymphocyte count on postoperative day 3. Inhibition of lymphocyte proliferation was maximal on postoperative day 1 (55% normalized to preoperative values) and was detectable until postoperative day 7, when it was 25%. Expression of T-cell activation antigens was decreased during the first postoperative week with a maximum on postoperative day 1 for CD71 (29%) and on postoperative day 3 for CD25 (49%). Intracellular production of cytokines by T cells was decreased only on postoperative day 1 (50% for IL-2, 29% for IFN-γ and 22% for TNF-α). Immune functions returned to presurgery values by day 14. A major surgical procedure severely inhibits lymphocyte proliferation and various T-cell functions up to 1 week postoperatively.

Introduction

Infection following major surgical procedures remains one of the most feared complications in modern surgery. Previous studies have shown that the higher susceptibility to infections after surgery is related to impairment of immune functions, caused by the surgical trauma itself [1–5]. Furthermore, there seems to be a relationship between the severity of surgical trauma and the degree of impairment of immune functions, which has been demonstrated in studies where open surgery was compared minimally with invasive surgery [6–9]. All of these studies have been performed in humans. Some investigators studied patients with cancerous diseases undergoing surgery [2–4], others studied patients with noncancerous diseases undergoing a variety of surgical procedures of different magnitude [5]. In the studies investigating minimally invasive surgery the results were compared with the same open surgical procedure, but not to nonoperated controls. In this heterogeneous population of patients it is not clear how concomitant factors like the underlying diseases of the patients influence the immune system and therefore the study results. In this study, we used an animal procedure, which is closely related to surgery in humans, to investigate the effects of surgery on immune functions with the advantage of having a very controlled and clearly defined setting. Transperitoneal nephrectomy served to mimic major surgery, comparable with other general abdominal surgery.

Instead of using delayed-type hypersensitivity responses, as used by other investigators [10,11], which is a relatively ill-defined way to measure T cell-related immune function, we used novel flow cytometry-based whole blood assays. With this technique we were able to

analyze different T-cell functions more precisely, whereas the studies described above where usually focusing only on one particular function. We used whole blood mitogen-stimulated assays, which we consider superior to assays using isolated T cells or peripheral blood mononuclear cells, because they better approximate components in the circulation and preserve cell–cell interactions [12–14].

Materials and methods

Animals

The study was approved by the Institutional Animal Care and Use Committee at Stanford University, a facility that is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care and registered with the United States Department of Agriculture. Wild caught, male cynomolgus monkeys (*Macaca fascicularis*) with a weight between 5 and 8 kg were purchased from Charles River Biomedical Research Foundation, Inc. (Houston, TX, USA). The animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals prepared by the National Research Council and published by the National Academy of Sciences (National Academy Press, Washington, DC, USA, 1996).

Animals were assigned to two study groups. Group I ($n = 8$) underwent unilateral transperitoneal nephrectomy, in preparation for subsequent transplantation in another study. Group II ($n = 6$) consisted of nonoperated animals, which served as controls.

Surgery

After sedation with ketamine (Burns Veterinary Supply, Inc., Vancouver, WA, USA) 10 mg/kg i.m., animals received a loading dose of midazolam 0.1 mg/kg i.v. (Roche Pharmaceuticals, Nutley, NJ, USA) followed by a bolus of propofol (Abott Laboratories, North Chicago, IL, USA) given to effect for intubation. Once intubated, animals were maintained on constant infusions of propofol 0.1 mg/kg/min and midazolam 0.35 μg/kg/min. The animals were left to breathe spontaneously without ventilator assist, but still received oxygen at 1.5–2.0 l/min through the endotracheal tube.

Median laparotomy was performed and the left kidney was removed intracapsularly after ligation of the vessels and the ureter. The abdomen was closed in three layers using absorbable sutures (Vicryl, Burns Veterinary Supply, Inc., Vancouver, WA, USA). Animals were allowed to recover overnight in an intensive care unit. About 30 ml/kg of lactated Ringer's solution was administered intravenously 6 h postoperatively. Buprenorphine was given at a dose of 0.01–0.03 mg/kg i.m. q 8–12 h to control pain. Animals were offered only water on the day of surgery and after that had free access to food and water.

Urine output and fluid intake were recorded daily for the first 14 postoperative days. Vital signs, appetite, attitude and amount, and consistency of feces were recorded daily during the entire postoperative period. After initial visual assessment, animals were sedated for blood collection with ketamine in a dose range of 5–10 mg/kg IM. Upon sedation, animals were weighed and assessed for hydration status and wound status. Blood was collected before surgery and on postoperative days 1, 3, 7 and 14 from the femoral vein. About 2 ml was anticoagulated with sodium heparin for analysis of immune functions and 2 ml with sodium ethylenediaminetetraacetic acid (EDTA) for hemogram. The control animals were sedated at the same time-points as the operated animals for blood collections.

Reagents

Culture medium (CM) was prepared using RPMI 1640 (Life Technologies, Inc., Rockville, MD, USA) supplemented with 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine (all from Sigma, St Louis, MO, USA). Concanavalin A (Con A; Vector Laboratories, Inc., Burlingame, CA, USA) was dissolved in CM at 1 mg/ml, filtered as sterile and stored at −70 °C. Phorbol 12-myristate 13-acetate (PMA) was dissolved in dimethyl sulfoxide (DMSO) as a stock solution of 100 μg/ml, while brefeldin A was dissolved in DMSO as a stock solution of 5 mg/ml (all reagents from Sigma). Ionomycin (ICN Biomedical, Inc., Costa Mesa, CA, USA) was dissolved in ethanol (Fisher Scientific, Pittsburgh, PA, USA) as a stock solution of 500 μg/ml. PMA, ionomycin, and brefeldin A were stored at −70 °C and working solutions were prepared in CM.

Concentrated phosphate-buffered saline (PBS) was purchased from Coulter Corporation (Miami, FL, USA) and dissolved in deionized water. Antihuman monoclonal antibodies [anti-CD3, anti-CD25, anti-CD71, anti-interleukin (IL)-2, anti-interferon (IFN)-γ, and antitumor necrosis factor (TNF)-α] as well as isotype controls (mouse $IgG1_\kappa$ and mouse $IgG2a_\kappa$) were purchased from PharMingen (San Diego, CA, USA). Antiproliferating cell nuclear antigen (PCNA) monoclonal antibodies (Clone PC10) were purchased from Dako Corporation (Carpinteria, CA, USA). Ribonuclease (RNase) A, propidium iodide, saponin, and sodium azide were purchased from Sigma. Red blood cell lysis buffer was made up daily by dissolving 8.29 g ammonium chloride, 1 g potassium bicarbonate, and 37.2 mg Na_2-EDTA (all from Sigma) in 1 l of deionized water (pH 7.2). Permeabilizing buffer contained 1%

heat-inactivated fetal calf serum (HyClone, Logan, UT, USA), 0.1% saponin, and 0.1% sodium azide in PBS. Formaldehyde solution (>36.5% in water) was purchased from Fluka Chemie AG (Buchs, Switzerland), and methanol was purchased from Fisher Scientific (Pittsburgh, PA, USA).

Whole blood mitogen-stimulated lymphocyte proliferation and T-cell function assays

To analyze the effect of surgery on lymphocytes, we used whole blood mitogen-stimulated lymphocyte proliferation and function assays developed in our laboratory and optimized for the use with whole blood from cynomolgus monkeys.

Assessment of lymphocyte proliferation and T-cell functions by flow cytometry

Whole blood was diluted 1:10 with CM. Con A (7.5 μg/ml) was added as mitogen stimulus. For each sample, 1000 μl of diluted blood was then aliquoted into each of two replicate wells of a 48-well cell culture cluster (Corning, Inc., Corning, NY, USA). The plates were then incubated at 37 °C in a humidified 5% CO_2-air incubator for 72 h.

After incubation, the samples were analyzed using an Epics XL-MCL flow cytometer equipped with an air-cooled argon-ion laser (488 nm) using SYSTEM II COULTER software (Coulter Corporation). To ensure comparability of fluorescence intensities on different days, the flow cytometer was calibrated daily with relatively low intensity fluorescent microbeads (Flow-Set microspheres, Coulter Corporation).

Assessment of lymphocyte proliferation by PCNA analysis

About an 8 ml of lysis buffer was mixed with 800 μl of diluted whole blood, and red blood cells were lysed for 5 min at room temperature. After washing with PBS, leukocytes were pelleted (200 g, 5 min) and resuspended in PBS-containing 1% formaldehyde for 5 min. Fixed cells were pelleted, washed once with PBS, and resuspended in cold methanol at 4 °C for 10 min. After washing with PBS, the cell pellet was resuspended in a staining mixture-containing 102 μl permeabilizing buffer, 10 μl RNase (100 mg/ml in water), 5 μl propidium iodide (1 mg/ml in water) and 7.5 μl fluorescein isothiocyanate (FITC)-labeled anti-PCNA monoclonal antibodies. Cells were incubated in the staining mixture for 25 min in a water bath at 37 °C. Before analysis, leukocytes were pelleted, washed with PBS, and resuspended in PBS-containing 10 μg/ml propidium iodide. Two-color analysis was performed by collecting the FITC signal (PCNA) through a 525 nm band-pass filter and the propidium iodide fluorescence emission through a 635 nm band-pass filter. Total lymphocytes were gated using forward and side light scatter [15]. The lymphocyte gate was set conservatively, to exclude as many dead cells and as much debris as possible. Proliferating cells were identified and enumerated in two-parameter DNA/PCNA distributions as $PCNA^+$ cells with S/G_2M-phase DNA content. Five thousand-gated lymphocytes were collected per sample. For simplicity, we refer to these data as percentage SG_2/M cells. Unstimulated blood was used as a negative control.

Assessment of T-cell surface antigen expression

Expression of the following surface-activation antigens on T cells was quantitated by flow cytometry: transferrin receptor (CD71) and IL-2 receptor-α chain (CD25). In order to perform three-color flow-cytometric analysis, the following monoclonal antibody combination was used: FITC-labeled anti-CD71 + phycoerythrin (PE)-labeled anti-CD3ε + Cy-Chrome-labeled anti-CD25. About 200 μl of diluted blood was washed with PBS and pelleted (200 g for 5 min); 5 μl of each of the monoclonal antibodies was added (in the combination listed above). After incubation in the dark for 30 min, 4 ml of lysis buffer was added to each tube and red blood cells were lysed at room temperature for 2 min. Leukocytes were pelleted, washed with PBS, and then resuspended in 500 μl PBS-containing 0.5% formaldehyde.

All samples were analyzed by flow cytometry within 6 h of preparation. Emitted light of the fluorochromes was collected through 525 nm (FITC), 575 nm (PE) and 675 nm (Cy-Chrome) band-pass filters, respectively. Forward and side scatter were used to differentiate lymphocytes from debris, dead cells, and other leukocytes. Five thousand light scatter gated lymphocytes were analyzed per sample. Unstimulated and stimulated diluted whole blood cultures were incubated with isotype control antibodies (PE-labeled mouse $IgG1_\kappa$ or $IgG2a_\kappa$) and used as specificity controls. To distinguish between fluorescence-positive and -negative cell populations, analysis regions were set using isotype controls to achieve nonspecific binding of <1% within the positive event regions. For all studied activation antigens, the entire population of lymphocytes was light scatter gated and $CD3^+$ cells were subsequently subgated. The percentages of $CD3^+$ lymphocytes expressing CD25, CD71 were calculated thereafter.

Assessment of intracellular T-cell cytokine production

For the detection of intracellular T-cell cytokines, 100 μl of undiluted whole blood was incubated in 4-ml polypropylene tubes for 5 h at 37 °C with PMA (30 ng/ml) and ionomycin (750 ng/ml) in the presence of brefeldin A (10 μg/ml, added 30 min after the mitogens). After incubation, 50 μl of whole blood was aliquoted into 4-ml polystyrene tubes (Applied Scientific, San Francisco, CA,

USA) and incubated with 3 μl of peridinin-chlorophyll *a* complex–Cy-Chrome (PerCP–Cy5.5)-labeled monoclonal antihuman CD3 antibodies for 15 min at room temperature in the dark. Subsequently, the cells were fixed and permeabilized using a standard fixation-permeabilization reagent kit and the manufacturer's immunofluorescence staining protocol (IntraPrep, Immunotech, Marseille, France). Cells were fixed by adding 100 μl of Reagent 1 (containing 5.5% formaldehyde) to each sample and incubated at room temperature in the dark for 15 min. After washing with PBS, cells were permeabilized by adding 100 μl Reagent 2 (containing saponin) to each sample 5 min before adding anticytokine monoclonal antibodies and incubating the samples at room temperature in the dark for 15 min. For staining of intracellular cytokines, 2 μl of FITC-labeled anti-IL-2, FITC-labeled anti-IFN-γ, and PE-labeled anti-TNF-α were used. Samples were analyzed by flow cytometry within 6 h of preparation. Emitted light of the fluorochromes was collected through 525 nm (FITC), 575 nm (PE) and 675 nm (PerCP–Cy5.5) band-pass filters, respectively. Forward and side scatter were used to differentiate lymphocytes from debris, dead cells, and other leukocytes. Five thousand light scatter gated lymphocytes were analyzed per sample. Unstimulated and stimulated diluted whole blood cultures were incubated with isotype control antibodies (PE-labeled mouse $IgG1_{\kappa}$) and used as specificity controls. To distinguish between fluorescence-positive and -negative cell populations, analysis regions were set using isotype controls to achieve nonspecific binding of <1% within the positive event regions. For all studied cytokines, the entire population of lymphocytes was light scatter gated and $CD3^+$ cells were subsequently subgated. The percentages of $CD3^+$ lymphocytes also positive for IL-2, IFN-γ, or TNF-α were calculated thereafter.

Statistical analysis

The immunosuppressive effect of surgery was quantified by expressing the parameter of interest (i.e. lymphocyte proliferation or activation or T-cell cytokine production) normalized to 100%, using the following formula:

$$\text{Proliferation (or activation or cytokine production)}(\%) = \frac{\text{'after surgery'}}{\text{'before surgery'}} \times 100$$

'Before surgery' represents the average result obtained from stimulated whole blood collected at three different time-points (baselines) before surgery, while 'after surgery' represents the results obtained from stimulated whole blood after surgery.

Statistical analysis were performed using the statistics program SPSS for Windows, version 10.0 (SPSS, Inc., Chicago, IL, USA). Values are shown as mean ± SEM. The nonoperated group and the operated group were compared using the Mann–Whitney *U*-test. A *P*-value ≤0.05 was considered significant.

Results

All animals tolerated the surgical procedure well. There were no complications related to anesthesia or surgery. In all cases the postoperative course was uneventful, especially there was no evidence for infection in either of the animals.

In the nephrectomy group surgery caused a decrease in absolute lymphocyte count in the peripheral blood. Normalized to values before surgery, average lymphocyte count was 25% lower on postoperative day 3. Lymphocyte count slowly reached preoperative values until postoperative day 14 (Fig. 1). Hematocrit did not decrease after surgery (data not shown).

Surgery was also associated with impaired lymphocyte proliferation and function. After stimulation with Con A *in vitro*, mean inhibition of lymphocyte proliferation in whole blood was maximal on postoperative day 1 (55% normalized to preoperative values) and was sustained until postoperative day 7, when it was 25% (Fig. 2). This was significantly different to the control group, where lymphocyte proliferation remained 100 ± 9%. No difference between the two groups could be detected by postoperative day 14.

Surgery also decreased expression of T-cell activation antigens (Fig. 3). Mean inhibition of CD71 was maximal on postoperative day 1 (29%) and was sustained until

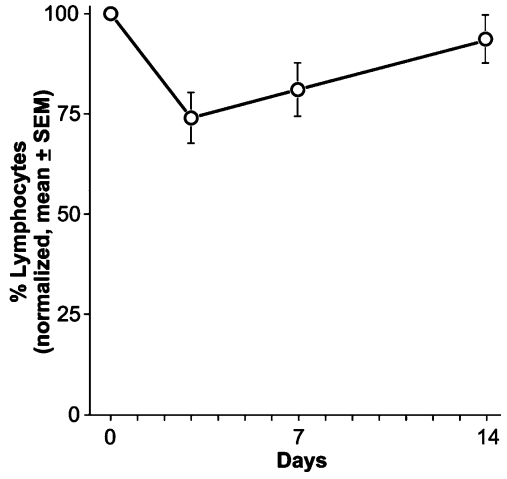

Figure 1 Absolute lymphocyte count after nephrectomy. Unilateral nephrectomy was performed in eight cynomolgus monkeys. Blood samples were obtained at different time-points after surgery. Absolute lymphocyte count is represented normalized to 100% (i.e. absolute lymphocyte count before surgery). Values are mean ± SEM.

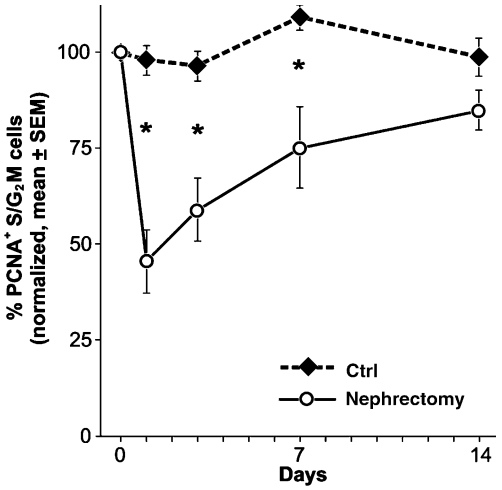

Figure 2 Inhibition of lymphocyte proliferation after nephrectomy. Unilateral nephrectomy was performed in eight cynomolgus monkeys. Six nonoperated animals were controls. Whole blood, which was obtained at different time-points, was stimulated with concanavalin A and incubated in culture medium for 72 h. Lymphocyte proliferation was assessed by flow-cytometric analysis of expression of proliferating cell nuclear antigen (PCNA) in cells in S/G_2M-phase. Inhibition of proliferation is expressed normalized to 100% (i.e. maximal proliferation after mitogen stimulation before surgery). Values are mean ± SEM. Asterisk (*) indicates $P < 0.05$ (Mann–Whitney *U*-test).

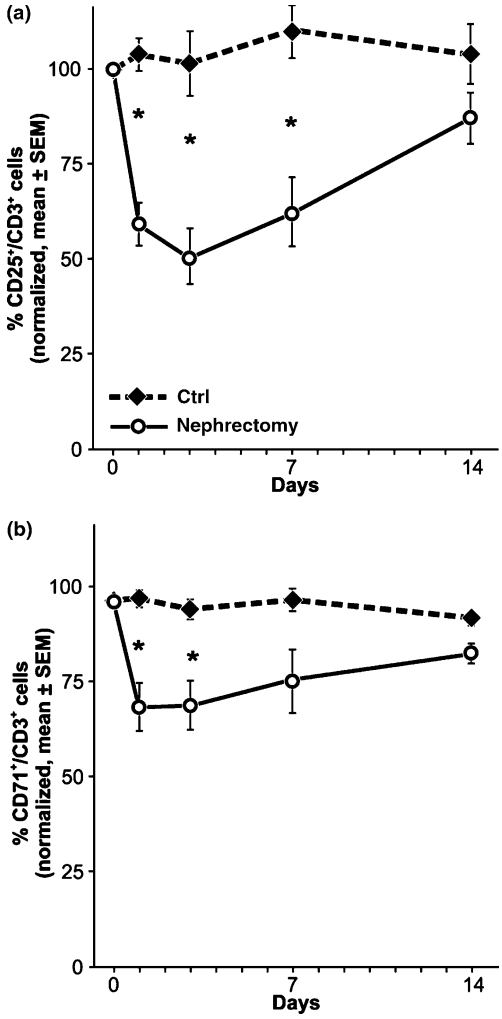

Figure 3 Inhibition of expression of T-cell surface antigens after nephrectomy. Unilateral nephrectomy was performed in eight cynomolgus monkeys. Six nonoperated animals were controls. Whole blood, which was obtained at different time-points, was stimulated with concanavalin A and incubated in culture medium for 72 h. Expression of different antigens on the surface of T cells was assessed by flow cytometry. T cells were identified by staining their surface for CD3, then flow cytometry determined the percentage of $CD3^+$ cells positive for interleukin (IL)-2 receptor-α chain (CD25; a) and transferrin receptor (CD71; b). Inhibition of surface antigen expression is represented normalized to 100% (i.e. maximal expression after mitogen stimulation before surgery). Values are mean ± SEM. Asterisk (*) indicates $P < 0.05$ (Mann–Whitney *U*-test).

postoperative day 3 (Fig. 3a). For CD25 maximal inhibition occurred on postoperative day 3 (49%) and was sustained until postoperative day 7, where it was 37% (Fig. 3b). This was also highly significantly different compared with the control group, where expression of T-cell activation antigens remained unchanged throughout the postoperative 2 weeks.

Finally, production of cytokines by T cells was lower among operated animals compared with control animals (Fig. 4). In response to stimulation with PMA and ionomycin, significantly fewer T cells in the operated group produced IL-2 and IFN-γ compared with the control group (operated animals, 50% IL-2, 71% IFN-γ; control animals, 106% IL-2, 101% IFN-γ; normalized to baseline values before surgery). This effect could only be observed on postoperative day 1. Later on, production of IL-2 and IFN-γ increased, reaching levels similar to those observed before surgery. For TNF-α a similar pattern was measured (operated animals, 78%; control animals, 102%), but the effect of surgery was less pronounced, and did not reach statistical significance between the two groups (Fig. 4c).

Discussion

This study shows a significant impairment of lymphocyte proliferation and various T-cell functions after a major surgical trauma, which is initiated with the surgical trauma and slowly recovers within the first postoperative week.

We could demonstrate that the total pool of circulating lymphocytes was decreased in the early postoperative phase, which is consistent with the results of Slade *et al.* [1]. This was not due to dilutional changes because the animals hematocrits did not decrease significantly after surgery. It is likely that apoptosis in circulating lympho-

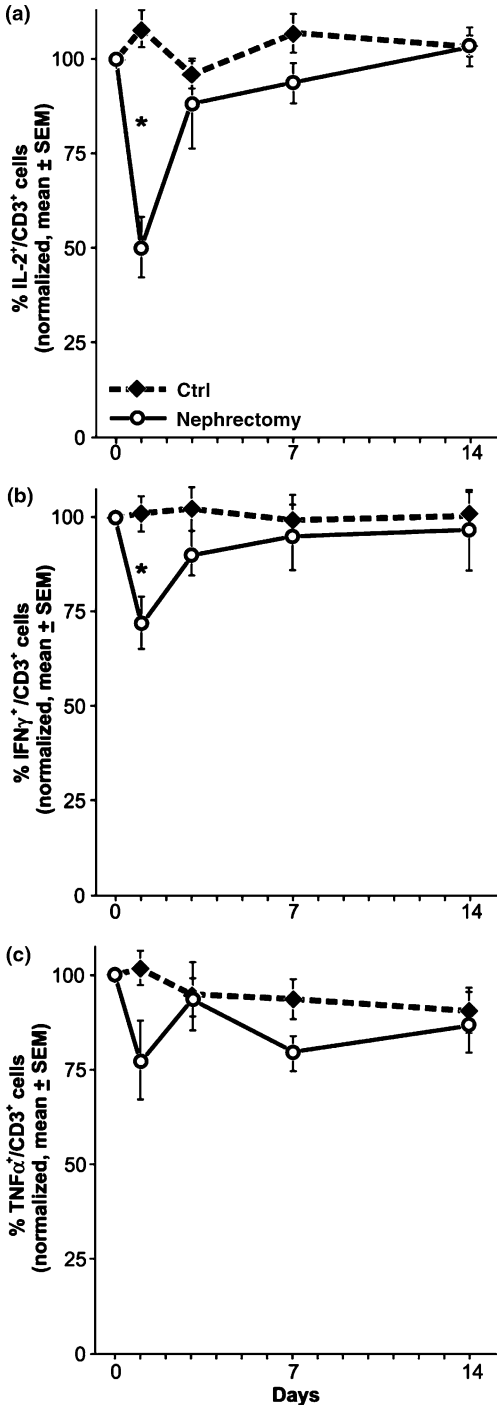

Figure 4 Inhibition of intracellular cytokine production after nephrectomy. Unilateral nephrectomy was performed in eight cynomolgus monkeys. Six nonoperated animals were controls. Whole blood, which was obtained at different time-points, was stimulated with phorbol 12-myristate 13-acetate and ionomycin in the presence of brefeldin for 5 h. Intracellular T-cell cytokine production was assessed by flow cytometry: T cells were identified by staining for CD3 and fixed with formaldehyde, then the cells were permeabilized with saponin and treated with monoclonal antibodies directed against cytokines [interleukin (IL)-2, interferon (IFN)-γ, tumor necrosis factor (TNF)-α]. Flow cytometry identified the percentage of $CD3^+$ cells positive for IL-2 (a), IFN-γ (b), or TNF-α (c). Inhibition of cytokine production is represented normalized to 100% (i.e. maximal production after mitogen stimulation before surgery). Values are mean ± SEM. Asterisk (*) indicates $P < 0.05$ (Mann–Whitney *U*-test).

cytes is responsible for the depletion of lymphocytes after surgery. Apoptosis is mediated by an enhanced expression of the Fas–Fas ligand system [16] and down-regulation of antiapoptotic factors, such as bcl-2 [17]. This unregulated activation of apoptosis seems to be initiated by oxidant stress and generation of reactive oxygen species caused by surgery [18].

Several T-cell functions were severely impaired. Production of cytokines like IL-2 and IFN-γ, and to a lesser extent TNF-α, was markedly decreased on postoperative day 1 and then recovered by postoperative day 3. Faist *et al.* [19] describe a possible mechanism for this finding. Prostaglandin E_2, which is more extensively produced by monocytes/macrophages after trauma, leads to an inhibition of IL-2 and IFN-γ production. Furthermore, it inhibits the expression of CD25, the IL-2 receptor-α chain. Horgan *et al.* [20] showed a diminished IL-2 production and mRNA expression after burn injury in humans and mice, but did not find a decrease in CD25 expression. In our study, we could clearly demonstrate that expression of CD25 was reduced during the first postoperative week. The interaction of IL-2 and CD25 is predominantly necessary for lymphocyte proliferation. We have shown that surgical trauma suppressed both, IL-2 production and expression of CD25. Consequently, the proliferation of lymphocytes was diminished.

In addition to CD25, we have shown that expression of CD71 was reduced in a similar pattern. CD71, transferrin receptor, is a widely distributed cell surface receptor on most proliferating cells [21]. Expression of transferrin receptor on the surface is up-regulated during T-cell activation [22] and it has been shown to mediate T-cell activation and proliferation in a functional relationship with the T-cell receptor [23]. Therefore, the reduction of transferrin receptor expression is another factor associated with a decrease in lymphocyte proliferation.

Because T cells are involved in cell-mediated and in humoral immunity, an impairment of T cells, as shown in our study, affects both cell-mediated and humoral immunity.

A finding that remains unclear is the relationship between the time courses of cytokine production versus lymphocyte proliferation and expression of surface-activation antigens of T cells after the surgical trauma. We found that inhibition of cytokine production was maximal on postoperative day 1 and thereafter was hardly

detectable. Inhibition of lymphocyte proliferation and expression of surface antigens were maximal on postoperative day 1 as well, but remained low during the first postoperative week. This discrepancy could be explained with the different assays. To assess the cytokine production, we used PMA and ionomycin to stimulate the lymphocytes *in vitro*, whereas for measuring lymphocyte proliferation and expression of surface antigens Con A was used. The combination of PMA and ionomycin acts directly on calcium metabolism and protein kinase C activity [24]. This may be a much stronger stimulus compared with other stimulation agents that act through the T-cell receptor, like Con A, or through co-stimulatory receptors [25,26]. In addition, blood was diluted 1:10 with CM in the proliferation assay, but not in the cytokine assay. Therefore, it seems possible that overall stimulation for lymphocyte activation was lower in the proliferation assay than in the cytokine assay. This would explain why only a strong inhibitory effect of the surgical trauma on postoperative day 1 could be measured with both assays, whereas the decreasing effect on the following days was only detected with the proliferation assay.

Although the animal procedure was chosen because animals were being prepared for subsequent transplantation in another study, it served to mimic major surgery, comparable with other general abdominal surgery. The use of healthy animals enabled us to exclude as many other factors that could have an effect on the immune system as possible. Many major surgical procedures in humans have to be performed for cancerous diseases. Therefore, most data about immunologic parameters after surgery have been gained from patients with malignant diseases. As there is abundant evidence that tumor development causes generalized immunodeficiency [27], these data do not solely reflect the effect of surgery on the immune system. However, there still remain some factors apart from the surgical trauma itself that could have an influence on the immune system. General anesthesia is one of these factors. Previous studies indicate that anesthesia has either no effect on lymphocyte proliferation [28] or that some anesthetics have an inhibitory effect which is limited to 48 h after anesthesia [29]. As the anesthetics in our study were not the same as in these studies, this question remains unclear but is of limited relevance, because surgery and anesthesia will always be used together, at least in major surgery.

Another influencing factor could be the treatment with opioids for pain medication. Acute and chronic treatment with morphine suppresses a variety of immune responses that involve all the major cell types in the immune system [30,31]. Two different pathways have been discussed, a central pathway through communication with the neuroendocrine system [32] and a direct pathway where opioids bind directly to receptors present on immune cells [33,34]. In our study, we used buprenorphine for pain treatment up to 48 h after surgery. This partial morphine agonist is binding to the μ-receptor, which is present on T cells. So it is likely that buprenorphine had an additional inhibitory effect on T-cell functions through the direct and the central pathway in our study. However, this effect is limited to the first postoperative day.

We conclude that a major surgical procedure severely inhibits lymphocyte proliferation and various T-cell functions up to 1 week postoperatively. The underlying cause is the surgical trauma itself but other factors as anesthesia and opioid pain therapy may contribute to the immunosuppression.

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