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Complement activation during OKT3 treatment: A possible explanation for respiratory side effects

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Complement activation during OKT3 treatment: A possible explanation for respiratory side effects. Respiratory side effects that sometimes occur during treatment with anti-CD3 MAb OKT3 might result from pulmonary sequestration of activated neutrophils. Therefore, we studied complement activation in relation to activation and pulmonary sequestration of neutrophils during antirejection treatment with OKT3. In each of nine patients studied, plasma C3a-desarg and C4b/c levels increased compared with pretreatment values already in the first sample taken 15 minutes after the first dose of OKT3 ($P < 0.05$), with peak values at 15 and 30 minutes, respectively. Levels of neutrophil degranulation product elastase (complexed to α1-antitrypsin) also increased already at 15 minutes after the first dose of OKT3 ($P < 0.05$), which is before elevated levels of the cytokines TNFα, IL-6 or IL-8 were detectable. In contrast, upon subsequent OKT3 administrations or in the control group treated with methylprednisolone, neither complement activation, cytokine release nor neutrophil degranulation occurred. In five studied patients treated with OKT3, pulmonary sequestration of radiolabeled granulocytes was observed from 3 until 15 minutes after the first dose of OKT3, together with peripheral blood granulocytopenia, which lasted at least 30 minutes. In conclusion, we demonstrate a simultaneous activation of complement and pulmonary sequestration of activated granulocytes immediately following the first dose of OKT3. These phenomena may be involved in the development of respiratory side effects complicating this therapy.

OKT3 is an IgG2a murine monoclonal antibody (MAb) directed against the CD3 molecule on T lymphocytes and is used in the prophylaxis and treatment of acute rejection of transplanted organs [1, 2]. The first dose of OKT3 causes clinical side effects including rigors, fever, headache, nausea and diarrhea starting usually within several hours after administration. These side effects have been associated with systemic release of cytokines, such as tumor necrosis factor-α (TNFα), interleukin-2 (IL-2), γ-interferon, and IL-6 [3–6], which parallel clinical side effects. In addition, respiratory symptoms ranging from mild dyspnea to severe respiratory distress may occur after the first OKT3 administration [7–9]. These side effects may also result from the release of cytokines such as TNFα [6, 10, 11]. However, respiratory symptoms generally develop within 30 minutes following the first dose of OKT3 [8, 9] which is before elevated levels of circulating cytokines are detected [3–6]. Since in a previous study we demonstrated activation of coagulation and fibrinolysis before an increase in levels of circulating cytokines was detected [12, 13], we hypothesized that mechanisms other than cytokine release may be involved in the development of side effects upon the first administration of OKT3. There is ample evidence that dyspnea and pulmonary edema, in relation to conditions other than treatment with OKT3, can be induced by complement activation products and/or complement activated neutrophils [14–18]. Furthermore, complement activation products themselves may induce cytokine release, thus potentiating their noxious activities [10, 19–21].

Therefore, we have investigated the possible role of complement activation in the pathogenesis of side effects caused by OKT3, by measurement of complement activation products C3a-desarg and C4b/c in the plasma of nine renal allograft recipients during the first three days of anti-rejection treatment with OKT3. Furthermore, we studied neutrophil activation by measurement of degranulation products elastase (in complex with α1-antitrypsin) and lactoferrin in relation to the aforementioned complement activation products and the cytokines TNFα, IL-6 and IL-8. As a control group we studied eight renal transplant recipients who were treated for acute rejection of the allograft with methylprednisolone (MPNS) only. To study the possible consequences of complement activation for the development of respiratory side effects, we monitored sequestration of ^{99m}Tc-hexamethylpropyleneamine-oxime (HMPAO) labeled granulocytes into the pulmonary vasculature in five of the OKT3 treated patients.

Methods

Patients

Nine renal allograft recipients (6 male, 3 female; median age 47 years, range 31 to 61 years) were studied who received OKT3, a murine IgG2a MAb directed against the CD3 complex (Ortho Diagnostic Systems, Raritan, New Jersey, USA) for the treatment of acute allograft rejection. Basic immunosuppressive drug therapy consisted of prednisolone (10 mg/day) and cyclosporin A (CsA; dosage based on whole blood trough

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levels; range 150 to 200 μg/liter during the first three months, 100 to 150 μg/liter thereafter). Whole blood parent compound CsA levels were measured with a commercially available radioimmunoassay (Cyclo-Trac; Incstar, Minnesota, USA). The diagnosis of acute rejection was based on clinical manifestations and confirmed by histological examination of a needle biopsy from the allograft. Overhydration, defined as an increase in body weight of more than 3%, was evaluated by assessing weight, fluid balance and a chest X-ray taken the day before treatment with OKT3. Three patients underwent pretreatment dialysis to correct fluid overload. One patient was treated with 500 mg furosemide before treatment with OKT3; there was no suspicion of fluid overload in the other five patients. Treatment with OKT3 consisted of a daily bolus injection of 5 mg OKT3 on ten consecutive days. The first dose of OKT3 in each patient was preceded by the infusion of 500 mg MPNS, over a period of 30 minutes just before OKT3 administration. In addition to MPNS, 25 mg promethazine was administered orally. During the first two days of treatment, blood samples were drawn just before medication, and at 15, 30 minutes, one, two, four and six hours after OKT3 administration. On the third day, samples were obtained before and at one and six hours after OKT3 administration. The control group consisted of eight renal allograft recipients (4 male, 4 female; median age 52 years, range 36 to 60 years) who were treated for acute allograft rejection with MPNS (500 mg/day on 6 consecutive days). Blood samples from this control group were obtained during the first three days of treatment, before and at one and six hours after administration of MPNS.

Blood was collected in siliconized vacutainer tubes to which EDTA (10 mM) and Polybrene (0.05%, wt/vol) were added to prevent any *in vitro* complement activation [22]. Plasma was collected immediately by centrifugation and was stored in multiple aliquots at −80°C.

Total leukocyte and differential counts were determined by flowcytometry (Technicon H1 system, Technicon Instruments, Tarrytown, Pennsylvania, USA) in blood anticoagulated with EDTA.

Assays for C3a-desarg and C4b/c

Plasma concentrations of C3a-desarg were measured with a radioimmunoassay (RIA) as described before [23]. The upper limit of values in plasma of 20 healthy controls, defined as mean + 2 SD, was 5 nmol/liter.

Plasma levels of C4 activation products were measured with a sandwich ELISA [24], using a murine MAb (anti-C4-1) directed against a neo-epitope exposed on C4 activation products C4b, C4bi, and C4c (further designated as C4b/c) which does not bind native C4, and a biotinylated rabbit polyclonal antiserum against human C4 (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). The detection limit of the assay is 0.025 nmol/liter and the intra- and interassay coefficients of variation are less than 11%. The specificity of the assay has been demonstrated by *in vitro* experiments in which classical or alternative complement activators were added to human serum. Results from these experiments showed that the assay only detects classical pathway activation [24]. Samples were related to a standard preparation that consisted of completely activated serum which contained 2000 nmol C4b/c per liter. The upper limit of values in plasma of 20 healthy controls, defined as mean + 2 SD, was 55 nmol/liter.

Assays for elastase-α1-antitrypsin and lactoferrin

Plasma levels of elastase-α1-antitrypsin complexes and lactoferrin were measured with radioimmunoassays (RIA's), as described before [25]. Samples were related to standard preparations of preformed elastase-α1-antitrypsin complexes or purified lactoferrin. The upper limit of values of elastase-α1-antitrypsin and lactoferrin in plasma of 20 healthy controls, defined as mean + 2 SD, was 3.9 nM and 5.3 nM, respectively.

Assays for TNF, IL-6 and IL-8

Plasma TNFα was measured with a commercially available ELISA (Medgenix, Billerica, Massachusetts, USA). The upper limit of values in plasma of 20 healthy controls, defined as mean + 2 SD, was 80 pg/ml.

Serum IL-6 was measured using the B9 bioassay [26]. Samples were related to a standard preparation of recombinant human (rh) IL-6. In control experiments the assay was not influenced by OKT3, CsA and MPNS concentrations occurring *in vivo* (data not shown). The upper limit of values in plasma of 20 healthy controls, defined as mean + 2 SD, was 13 pg/ml.

Plasma IL-8 was measured with a sandwich ELISA, using a coating of murine Mab (anti-IL-8 Mab1) and a conjugate consisting of a biotinylated monoclonal antibody (anti-IL-8 Mab s-12-14) [27]. The upper limit of values in plasma of 20 healthy controls, defined as mean + 2 SD, was 45 pg/ml.

Labeling and imaging of granulocytes

All procedures were performed with sterile, pyrogen-free glassware and reagents. From each patient, who was to be treated with OKT3, 46 ml fresh venous blood was collected and suspended into 10 ml acid citrate dextrose and 4 ml plasma. After sedimentation of erythrocytes for 45 minutes, supernatant was collected and centrifuged at 200 g for 10 minutes. The pellet of leukocytes was resuspended in 3 ml plasma and centrifugated over a two-step Percoll gradient. Granulocytes were collected, resuspended in NaCl 0.9% and incubated for 20 minutes at 20°C with 925 MBq of ^{99m}Tc HMPAO (Amersham, Amersham, UK), which was prepared as described [28]. After centrifugation the cell pellet was resuspended in 2 ml NaCl 0.9% and reinjected into the patient. The administered dose varied between 300 and 500 MBq, depending on the number of collected granulocytes. After two hours, when nonspecific pulmonary sequestration of labeled granulocytes had vanished, infusion of MPNS was started. During MPNS infusion ^{99m}Tc HMPAO granulocyte counts over the lungs were monitored. Then, the first intravenous bolus injection of 5 mg OKT3 was administered.

We monitored the sequestration of ^{99m}Tc HMPAO labeled granulocytes into the pulmonary vasculature during the first 30 minutes after the first OKT3 administration in five of eight patients treated with OKT3. Since pulmonary sequestration of ^{99m}Tc HMPAO labeled granulocytes was monitored during the period of MPNS administration, these patients served as their own controls for the effects of MPNS administration on pulmonary accumulation of granulocytes. Since most patients experienced rigors and fever, usually starting from 45 to 60 minutes after the first OKT3 administration, we did not extend the monitoring period beyond 30 minutes. Sequestration of labeled

granulocytes, and cell numbers in the peripheral blood were calculated as the percentage relative to pretreatment counts.

Each patient sat in a semi-upright position in front of a large field of view gamma camera (Siemens, Germany) mounted with a low energy collimator. Counts per pixel were plotted against time. Images were acquired in 60 seconds time frames on a DEC/PDP11/23 computer system.

In vitro *complement activation by OKT3*

The ability of OKT3 to bind C3b on T cells was determined by FACS® analysis (Becton and Dickinson, Mountain View, California, USA). Heparinized blood was collected from three healthy subjects. Mononuclear cells (PBMC) were separated by centrifugation on Ficoll-Paque (density 1.076; Pharmacia, Uppsala, Sweden). PBMC were incubated for 30 minutes at 37°C with 1 μg/ml FITC-labeled OKT3 (Ortho Diagnostic Systems), that is, at a similar concentration as the plasma peak level occurring shortly after the first dose of OKT3 [29], in the presence of fresh autologous serum. Control experiments included incubation with heat inactivated serum (45 minutes at 56°C) or fresh serum containing EDTA (10 mM final concentration). After incubation with FITC-labeled OKT3 and serum, cells were incubated with a biotinylated MAb directed against a neo-epitope on C3b, C3bi and C3c (anti-C3-28) [30], followed by incubation with streptavidin-PE (Beckton Dickinson).

In vitro *studies on OKT3-mediated aggregation of granulocytes and lymphocytes*

Heparinized blood and serum were obtained from three healthy volunteers. Granulocytes were purified from the heparinized blood as described [31], resuspended in incubation medium [32] and kept at room temperature. Plastic culture bottle nonadherent PBMC, further referred to as lymphocytes, were collected, resuspended in incubation medium and kept at room temperature. These cell suspensions always contained less than 1% monocytes (defined as CD14 positive cells).

Aggregation studies of granulocytes and lymphocytes were performed under continuous stirring in siliconized special glass cuvettes (Hellma GmbH & Co., Müllheim, Germany) at 37°C [32]. After mixing of equal volumes of the granulocyte and lymphocyte suspensions, each containing 5×10^9 cells/ml, the aggregation stimulus was added, consisting of either: (1) fresh autologous serum containing a concentration of OKT3 that resulted in a final concentration of 1 μg/ml of OKT3 in the cell-suspension; (2) heat-inactivated autologous serum with the same concentration of OKT3 as in 1; or (3) fresh autologous serum containing a concentration of an IgG2a control MAb directed against TNP antigens without reactivity with human blood cells or immunoglobulins (Central Laboratory of the Blood Transfusion Service) resulting in a final concentration of 1 μg/ml MAb in the cell-suspensions. From the cell-suspensions cytospin slides were made, fixed and stained with MayGrünwald/Giemsa. Slides were examined by light microscopy.

Statistical analysis

Values are presented as mean ± SEM. Statistical analysis was performed with the Wilcoxon signed ranks test. A P value less than 0.05 was considered to be significant.

Results

Among the nine patients treated with OKT3, three experienced dyspnea, starting in two of these patients within ten minutes after the first dose of OKT3, in the third patient at one hour. In contrast, none of the eight patients treated with MPNS suffered from dyspnea. There were no significant differences in pre-treatment clinical and laboratory parameters (sex, age, pretreatment weight vs. dry weight, granulocyte and lymphocyte counts, previous hemodialysis and treatment with furosemide) of those OKT3 treated patients who did and those who did not develop respiratory side effects. Overhydration was always corrected before the first administration of OKT3.

Complement activation in vivo

In all OKT3-treated patients, plasma C3a-desarg levels increased, from 6.1 ± 1.0 nmol/liter (mean ± SEM) before the first dose of OKT3 to a peak level of 22.9 ± 2.8 nmol/liter in the first sample taken at 15 minutes ($P < 0.05$; Fig. 1). C3a-desarg levels declined rapidly but remained significantly elevated as compared to pre-treatment values until one hour after the first dose of OKT3. C4b/c levels in plasma increased from 57 ± 6 nmol/liter before the first OKT3 dose to a peak level of 119 ± 12 nmol/liter at 30 minutes ($P < 0.05$). These levels remained significantly increased until two hours after the first dose of OKT3.

Elevated plasma levels of C3a-desarg or C4b/c were detected neither after OKT3 administration on the second and third day of treatment (Fig. 1) nor in the control group of patients treated with MPNS only (data not shown). Although the sampling frequency in the latter group of patients included less time points than in the OKT3 treated group (as described in **Methods**), plasma samples taken at 60 minutes after the first OKT3 administration still contained significantly elevated levels of C3a-desarg and C4b/c (Fig. 1), whereas the control group of MPNS treated patients had normal levels. Therefore, we consider it unlikely to have missed early activation of complement in the MPNS-treated patient group. In the OKT3 treated patients there was no significant correlation between the extent of complement activation and the development of respiratory side effects.

The results indicate activation of the classical complement pathway in the patients receiving OKT3. To exclude complement activation by aggregates in the OKT3 preparations, we incubated various concentrations of OKT3 (in a concentration range from 0 up to 100 μg/ml) with fresh serum, and determined C3a-desarg concentrations in the mixtures. The spontaneous complement activation measured in the presence of OKT3 was not different from that when PBS was added instead of OKT3, and was less than 5% of C3a-desarg levels that were measured when aggregated IgG was added (data not shown), excluding complement activation by OKT3-aggregates.

Neutrophil degranulation and cytokine release in vivo

From the group of nine patients that were treated with OKT3, eight showed a similar response to the first dose of OKT3. One patient (male, age 38 years) who was treated with OKT3 showed a distinct response to the first OKT3 administration and will, therefore, be discussed separately. In the group of eight

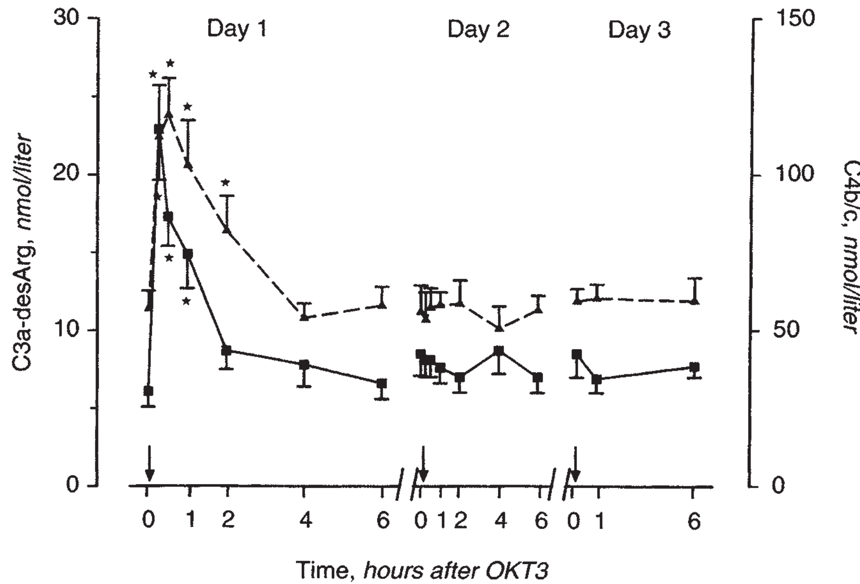


Fig. 1. *Plasma C3a-desarg (■) and C4b/c (▲) levels in nine patients during treatment with OKT3.* Results are shown as mean (± SEM) for 9 patients during the first 3 days of treatment with OKT3. Arrows indicate the daily administration of OKT3. Time points shown are: before, and at 15, 30, 60, 120, 240, 360 minutes after OKT3 administration on the first 2 days; before, and at 60 and 360 minutes after OKT3 administration on the third day. Significant increases ($P < 0.05$) compared to pre-treatment levels are indicated by an asterisk (Wilcoxon Signed Ranks Test).

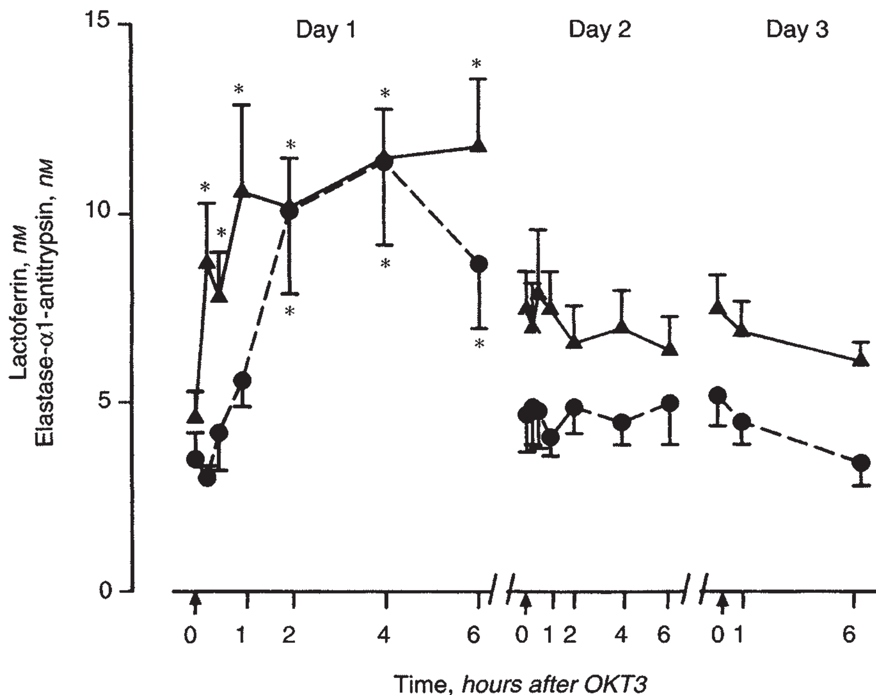


Fig. 2. *Plasma elastase-α1-antitrypsin (▲) and lactoferrin (●) levels in eight patients during treatment with OKT3.* Arrows indicate the daily administration of OKT3. Results are shown as mean values (± SEM) for eight patients during the first 3 days of treatment with OKT3. For further explanation of this figure see legend to Figure 1.

patients, plasma levels of elastase-α1-antitrypsin significantly increased from 4.6 ± 0.7 nM before OKT3 administration to 8.7 ± 1.6 nM, already in the first sample taken 15 minutes after the first dose of OKT3 ($P < 0.05$; Fig. 2). Levels increased more gradually after one hour and reached their maximum of 11.8 ± 1.8 nM at six hours. Plasma lactoferrin levels, which amounted 3.5 ± 0.7 nM before the first dose of OKT3, were not elevated until one hour and were first significantly increased (10.1 ± 2.2 nM) at two hours ($P < 0.05$), reaching their peak of 11.4 ± 2.2 nM at four hours. For both elastase-α1-antitrypsin and lactoferrin no significant changes were found after the second and third dose of OKT3.

Before the first administration of OKT3, plasma levels of TNF were 75 ± 13 pg/ml. Compared with pretreatment values, plasma TNF levels were first significantly elevated at half an hour after the first dose of OKT3 (325 ± 64 pg/ml), and reached peak levels at one hour (516 ± 76 pg/ml). They remained significantly increased until six hours. Upon subsequent administrations of OKT3, plasma TNF levels did not increase. Figure 3 shows that serum IL-6 levels significantly increased from 26 ± 7.6 pg/ml before OKT3 administration to a peak of 130 ± 34.7 pg/ml at two hours after the first administration of OKT3 ($P < 0.05$). The plasma level of IL-8 increased from 25.9 ± 4.9 pg/ml before treatment to 1111 ± 210 pg/ml at two hours after the first

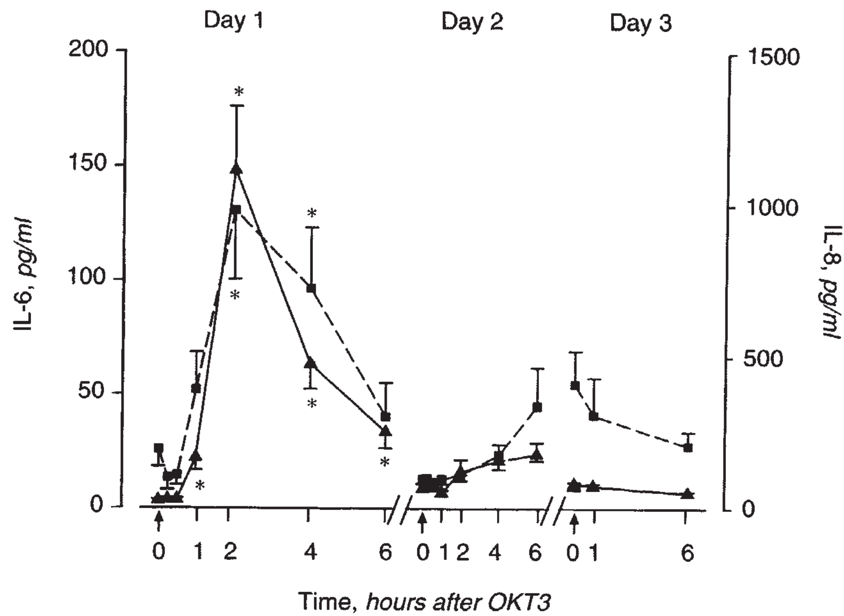


Fig. 3. *Plasma IL-6 (■) and IL-8 (▲) levels in eight patients during treatment with OKT3.* Results are shown as mean values (± SEM) for eight patients during the first 3 days of treatment with OKT3. For further explanation of this figure see legend to Figure 1.

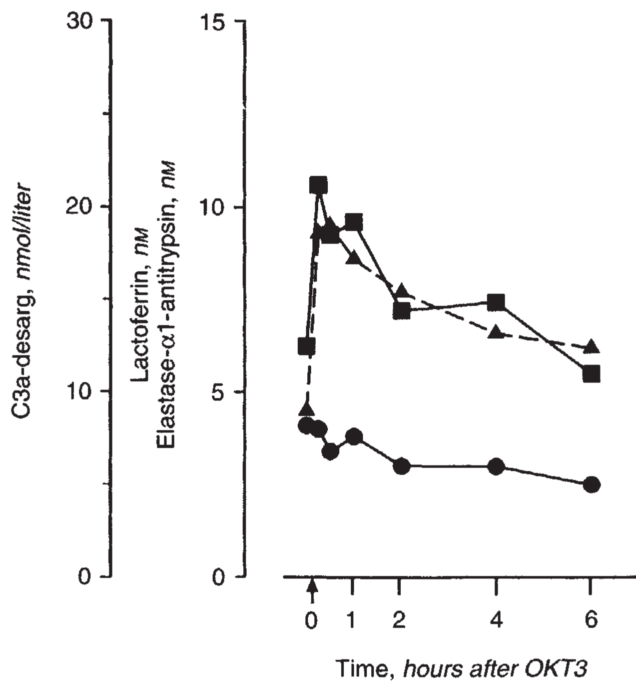


Fig. 4. *Plasma C3a-desarg (■), elastase-α1-antitrypsin (▲) and lactoferrin (●) in one patient without elevation of circulating cytokine levels after the first dose of OKT3.* The arrow indicates administration of OKT3. Timepoints shown are: before and at 15, 30, 60, 120, 240, 360 minutes after the first OKT3 administration.

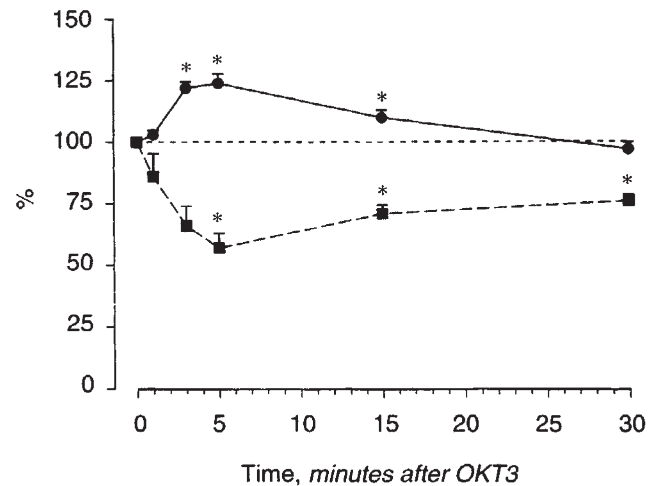


Fig. 5. *Sequestration of ^{99m}Tc HMPAO labeled granulocytes in the pulmonary vasculature.* Results are shown as mean (± SEM) for 5 OKT3 treated patients. Pulmonary sequestration of ^{99m}Tc HMPAO labeled granulocytes (●) and the number of granulocytes in the peripheral blood (■) were calculated as the percentage relative to pretreatment values. Before administration of OKT3, mean pulmonary counts of labeled granulocytes (± SEM) mounted 199.4 ± 54.3 counts per pixel; the number of granulocytes in the peripheral blood mounted 6.6 ± 1.0 × 10^9/liter. Significant changes ($P < 0.05$) compared to pretreatment levels are indicated by an asterisk (Wilcoxon Signed Ranks Test).

OKT3 administration ($P < 0.05$). For IL-6 and IL-8, no significant increases were observed upon subsequent OKT3 administrations. Although the actual values of circulating cytokines varied considerably, as is reflected by the SEM, the kinetics of these parameters within each of these eight OKT3 treated patients were comparable.

In the control group of eight patients treated for acute rejection of the renal allograft with MPNS only, no increases were found in circulating elastase-α1-antitrypsin, lactoferrin, TNF, IL-6 or IL-8 (data not shown).

The one patient that showed a distinct response to the first dose of OKT3 did not show any increase in plasma levels of TNF, IL-6 or IL-8 during the first day of treatment with OKT3. Administration of OKT3 was proven by detection of therapeutic levels of OKT3 in the plasma samples. In this patient, the first dose of OKT3 did induce complement activation, since plasma content of complement activation product C3a-desarg

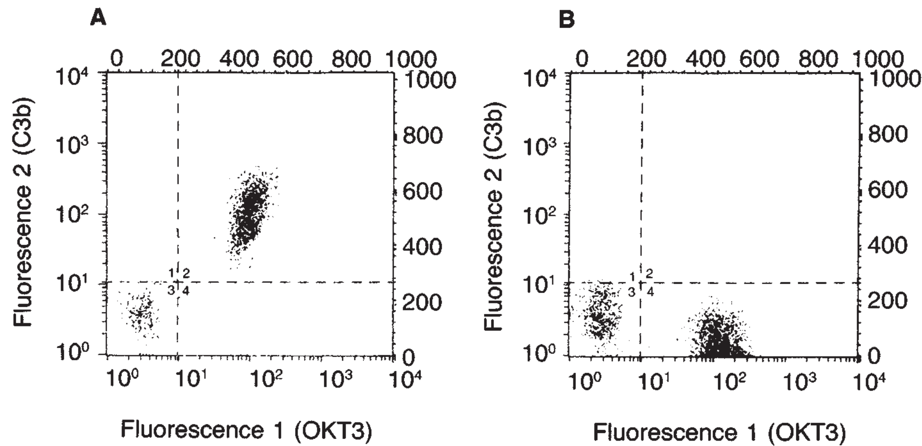


Fig. 6. *C3b deposition on OKT3 positive lymphocytes.* FITC-labeled OKT3 (1 μg/ml) and a biotinylated monoclonal antibody (second step: streptavidin-PE) against C3b (anti-C3-28) were used to detect C3b fixation on T cells. **A.** Virtually all OKT3 positive cells incubated with 10% fresh autologous serum fixed C3b. **B.** Absence of C3 fixation when heat-inactivated serum was used. Lymphocytes were gated on forward and sideward scatter.

increased similarly to that in the other eight patients (Fig. 4). Remarkably, plasma elastase-α1-antitrypsin content showed an increase at 15 and 30 minutes after the first dose of OKT3, comparable to that observed in the other eight OKT3 treated patients. In contrast, this increase in plasma elastase-α1-antitrypsin level was immediately followed by a continuous and steady decline. In sharp contrast to the findings in the other eight OKT3 treated patients, plasma levels of lactoferrin did not increase at all in this patient.

Sequestration of granulocytes in the lung

No pulmonary sequestration of ^{99m}Tc HMPAO granulocytes occurred in any patient during infusion of MPNS before the first dose of OKT3. However, sequestration of ^{99m}Tc HMPAO labeled granulocytes in the pulmonary circulation occurred in each of five patients and, as compared to pre-treatment values, was significant at three minutes after the first dose of OKT3 ($P < 0.05$), reaching its maximum of 126 ± 4.2% at five minutes. Sequestration of ^{99m}Tc HMPAO granulocytes in the lungs remained significantly elevated until 15 minutes and returned to pretreatment values at 30 minutes (Fig. 5). Pulmonary accumulation of granulocytes coincided with a fall in circulating granulocytes reaching a nadir of 57% ± 6.1 at five minutes. The percentage of circulating granulocytes remained decreased until 30 minutes after the first dose of OKT3 ($P < 0.05$).

To study possible mechanisms of granulocyte retention in the pulmonary vasculature, we performed several *in vitro* experiments on complement mediated interactions between lymphocytes and granulocytes.

Complement mediated aggregation of lymphocytes and granulocytes in vitro

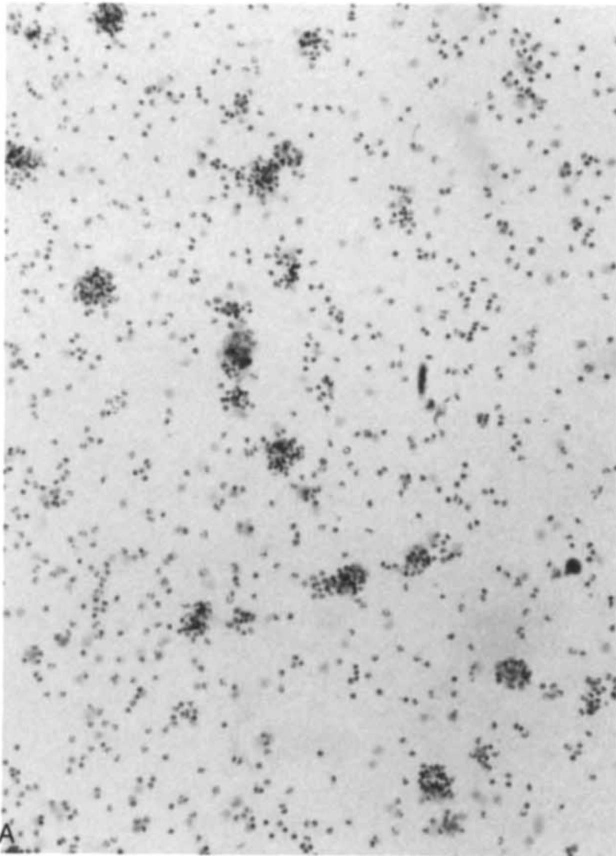
Figure 6 shows the results of a representative FACS experiment on *in vitro* C3b fixation by OKT3 coated T cells. Upon incubation with 10% (vol/vol) fresh autologous serum, C3b was fixed on virtually all T cells (Fig. 6a). This was neither observed with 10% (vol/vol) heat-inactivated serum (Fig. 6b), nor in the presence of EDTA (data not shown). The biological significance of this complement fixation on T cells is shown in Figure 7a. When fresh autologous serum and OKT3 were added to a mixture of granulocytes and lymphocytes, aggregation between granulocytes and lymphocytes occurred. This aggregation did not occur when heat-inactivated serum was added instead of fresh serum (Fig. 7b), or with the control IgG2a MAb (data not shown).

Discussion

Murine MAbs of the IgG2a subclass are able to activate human complement *in vitro* [33]. Therefore, complications occurring upon administration of murine MAbs to humans may be caused by complement activation products. Surprisingly, in the context of OKT3 administration to humans, only two studies have been reported on complement activating properties of OKT3 *in vitro* [34, 35] and—to the best of our knowledge—no prior studies have been published on *in vivo* complement activation by OKT3 in humans. Presumably, this is due to the general belief that murine MAbs only weakly activate human complement [36].

Current research on side effects caused by treatment with OKT3 focuses on cytokines as possible causative agents [3–6]. In mice and in humans, pre-administration of high dose methylprednisolone reduced both anti-CD3-induced cytokine release and toxic side effects [37, 38]. However, a recent study by Zlabinger et al in renal transplant recipients on side effects of OKT3 and the IgG2b murine monoclonal antibody BMA031, which is directed against the α/β T cell receptor, indicated that elevated circulating levels of cytokines cannot produce morbidity by itself [39]. In addition, respiratory side effects and hypotension that occur early after the start of treatment with OKT3 [8, 9] remain largely unexplained [40], although cytokines such as TNFα may be involved [6, 10, 11]. Since dyspnea and pulmonary edema in relation to conditions other than OKT3 treatment have been shown to be induced by complement activation products [14–18], the aim of the present study was to delineate the role of complement in the pathogenesis of clinical side effects of treatment with OKT3.

In this study we provide evidence that the first administration of OKT3 activates human complement *in vivo*. Subsequent doses of OKT3 do not activate complement most likely because the target for this anti-CD3 monoclonal antibody, namely circulating T lymphocytes, are then no longer present [29]. We also show that this activation occurs via the classical pathway and coincides with temporary sequestration of granulocytes in the pulmonary vasculature and disappearance of granulocytes from the circulation. Furthermore, we demonstrate that neutrophils are indeed activated immediately after the first dose of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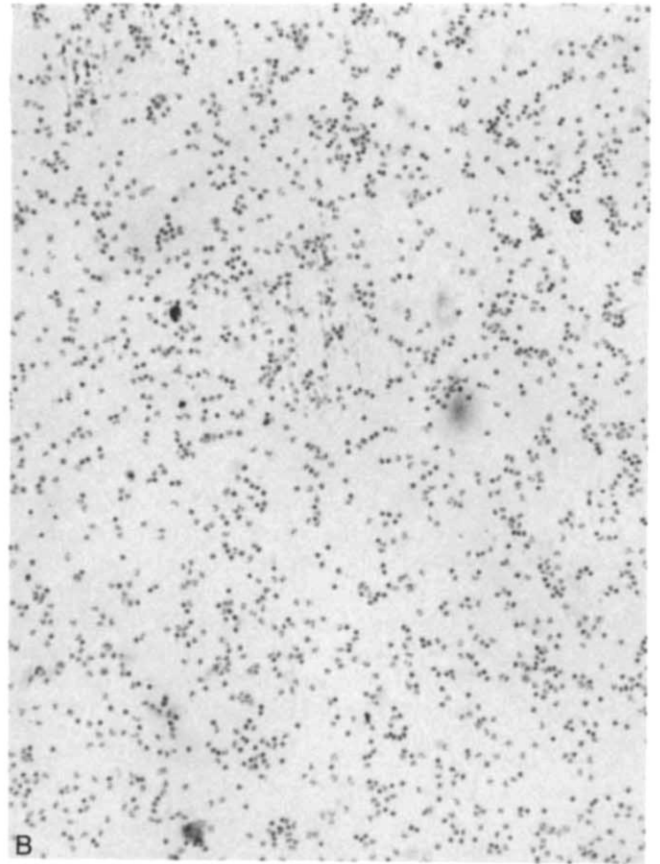
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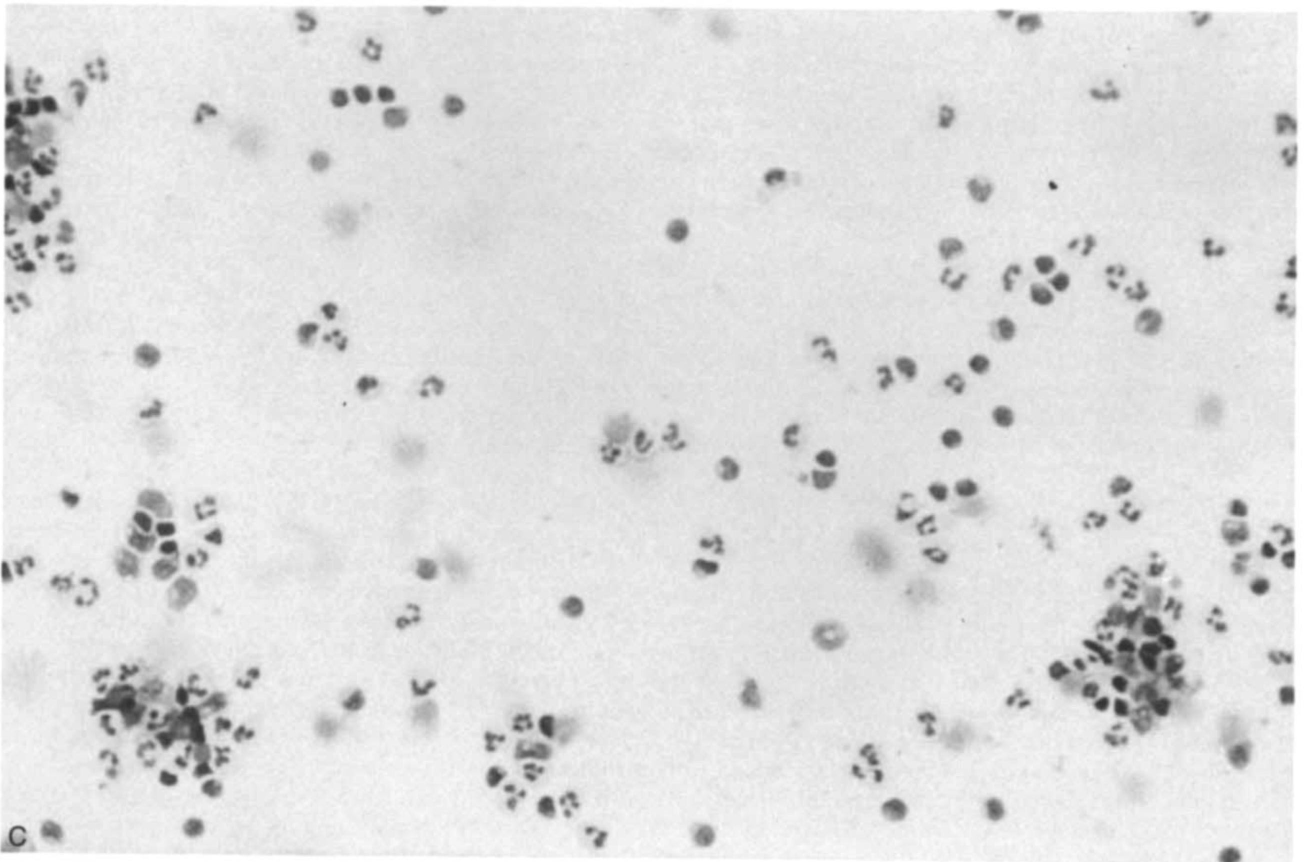
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Fig. 7. In vitro *aggregation of granulocytes and lymphocytes by OKT3 and autologous serum.* **A.** Aggregation of granulocytes and lymphocytes upon addition of OKT3 (1 μg/ml) and fresh autologous serum as complement source. **B.** This aggregation did not occur when heat-inactivated serum was used instead of fresh serum. **C.** A high power photomicrograph of an aggregate of lymphocytes and granulocytes.

OKT3, which is probably at least in part induced by complement activation products [41–43].

In vitro C5a induces degranulation of both azurophilic granules which contain elastase, and specific granules which contain lactoferrin [43, 44]. In sepsis patients plasma levels of elastase-α1-antitrypsin as well as lactoferrin are increased, but only the former appeared to correlate with plasma C3a-desarg levels [25], suggesting a role for complement in the release of azurophilic granules in sepsis. We propose that complement activation is responsible for the early release of elastase in the OKT3 treated patients for the following reasons: plasma C3a-desarg levels showed a fourfold increase as compared to pre-treatment values already at 15 minutes after the first dose of OKT3. At that moment no release of TNF, IL-6 or IL-8 had yet occurred. Therefore, complement activation products were in fact the earliest agonist for degranulation of neutrophils that were detectable in the circulation. In addition, in the single patient who did not show any increase in circulating cytokines in response to the first dose of OKT3, but who did show complement activation, an early increase in plasma elastase-α1-antitrypsin levels was indeed observed. However, this increase was not followed by a sustained elevation of elastase-α1-antitrypsin levels as was observed in the other OKT3 treated patients, and plasma levels of lactoferrin did not increase at all. This indicates the necessity of cytokine release for sustained activation of neutrophils.

Additional mediators that may be responsible for activation of neutrophils following the first dose of OKT3 include the cytokines TNFα [45, 46] and IL-8 [47, 48]. An increase in circulating TNF levels has been described in several reports on side effects caused by OKT3 [3, 4] and is also found in our study. We also demonstrate that this increase of plasma TNF levels is followed by a significant elevation of serum IL-6 and IL-8 levels with peak levels at two hours after the first dose of OKT3. This cytokine release probably results from OKT3 activated T cells [6], but may also be due to stimulation of monocytes by Fc-receptor crosslinking [49, 50]. In addition, release of IL-6 and IL-8 may have been induced by the elevated circulating levels of TNF [47, 48].

In vitro, both TNF [45, 46] and IL-8 [47] can induce degranulation of both azurophilic and specific granules of neutrophils. In other *in vivo* studies, in which healthy subjects were challenged with either rh-TNFα [51] or endotoxin [52], elevated plasma levels of both degranulation products from neutrophils were shown in the presence of elevated levels of circulating TNF and IL-6. Some additional evidence that release of cytokines contributes to the sustained activation of neutrophils during treatment with OKT3 was obtained from the one patient in whom, in the absence of elevated levels of circulating cytokines, plasma lactoferrin levels did not change at all, whereas plasma elastase-α1-antitrypsin levels were only increased during the first two hours after the first dose of OKT3. This was in sharp contrast with the findings obtained in the other eight patients treated with OKT3.

In the present study we also demonstrate immediate sequestration of granulocytes in the lungs upon the first dose of OKT3. Pulmonary accumulation of granulocytes may have been caused by adhesion of C5a activated neutrophils to the vascular endothelium [41, 42] and/or sequestration of cell-aggregates into the pulmonary vasculature. Indeed we demonstrated *in vitro* immunocytoadherence of granulocytes and lymphocytes upon addition of OKT3 and complement, which may suggest that aggregates of these cells are also involved *in vivo*. Our findings suggest that complement activation and subsequent pulmonary sequestration of activated granulocytes may be involved in the development of acute respiratory side effects that typically occur only after the first dose of OKT3. Three of nine patients treated with OKT3 became dyspneic despite preadministration of MPNS and careful prevention of fluid overload. The extent of complement activation or pulmonary accumulation of granulocytes in these three patients was not different from the other six OKT3 treated patients. Some subclinical respiratory distress may have occurred in these other patients, since a decrease in measured arterial PO_2 was reported by Büsing et al in 16 out of 18 patients; in the majority of cases (12 out of 16) this occurred as soon as 15 minutes after the first dose of OKT3 [9]. Although the present study does not prove that neutrophils migrate into the pulmonary interstitium, even without interstitial migration the pulmonary vascular endothelium may have been damaged by azurophilic degranulation products of the sequestered neutrophils. To demonstrate migration of activated neutrophils into the pulmonary interstitium or the alveolar space, bronchoalveolar lavage studies would be required. However, this would be too heavy a burden for patients receiving OKT3.

Occasionally, patients may develop more severe and even life threatening respiratory distress during treatment with OKT3 [7–9]. Our data do not allow definite conclusions whether this condition is solely due to complement activation and pulmonary sequestration of neutrophils, or if the release of cytokines such as TNFα is also involved [3–5]. Clinical and experimental studies on the pathogenesis of adult respiratory distress syndrome have indicated that this syndrome may result from direct toxic effects of complement and/or cytokines on pulmonary endothelial cells [10, 11], or from endothelial damage brought about by activated neutrophils [14–18]. Since all the abovementioned agents are present upon the first dose of OKT3, they will probably execute their noxious effects in concert with each other.

In conclusion, we demonstrate complement activation in patients upon administration of the first dose of OKT3 and provide *in vitro* evidence in support of the hypothesis that this activation has been caused by OKT3-coated T cells. Furthermore, we show immediate degranulation and sequestration of granulocytes in the pulmonary vasculature upon the first administration of OKT3. We suggest that these phenomena may play a role in the development of (immediate) respiratory side effects during treatment with OKT3. However, further work is needed to study the role of complement activation, relative to

that of other mediators, in the development of pulmonary symptoms after OKT3 administration.

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References

1. ORTHO MULTICENTER TRANSPLANT GROUP: A randomized clinical trial of OKT3 monoclonal antibody for acute rejection of cadaveric renal transplants. Ortho Multicenter Transplant Study Group. *N Engl J Med* 313:337–342, 1985
2. VIGERAL P, CHKOFF N, CHATENOUD L, CAMPOS H, LACOMBE M, DROZ D, GOLDSTEIN G, BACH JF, KREIS H: Prophylactic use of OKT3 monoclonal antibody in cadaver kidney recipients. Utilization of OKT3 as the sole immunosuppressive agent. *Transplantation* 41:730–733, 1986
3. ABRAMOWICZ D, SCHANDENE L, GOLDMAN M, CRUSIAUX A, VEREERSTREATEN P, DE PAUW L, WYBRAN J, KINNAERT P, DUPONT E, TOUSSAINT C: Release of tumor necrosis factor, interleukin-2 and gamma-interferon in serum after injection of OKT3 monoclonal antibody in kidney transplant recipients. *Transplantation* 447:606–608, 1989
4. CHATENOUD L, FERRAN C, LEGENDRE C, THOUARD I, MEROTE S, REUTER A, GEVAERT Y, KREIS H, FRANCHIMONT P, BACH JF: *In vivo* cell activation following OKT3 administration. *Transplantation* 49:697–702, 1990
5. BLOEMENA E, TEN BERGE RJM, SURACHNO S, WILMINK JM: Kinetics of interleukin-6 during OKT3 treatment in renal allograft recipients. *Transplantation* 50:330–331, 1990
6. FERRAN C, SHEENAN C, DY M, SCHREIBER R, MERITE S, LANDAIS P, NOEL LH, GRAU G, BLUESTONE J, BACH JF, CHATENOUD L: Cytokine related syndrome following injection of anti-CD3 monoclonal antibody: Further evidence for transient *in vivo* T cell activation. *Eur J Immunol* 20:509–515, 1990
7. ROWE PA, ROCKER GM, MORGAN AG, SHALE DJ: OKT3 and pulmonary capillary permeability. *Br Med J* 295:1099–1100, 1987
8. THOMAS DM, NICHOLLS AJ, FEEST TG, RIAD H: OKT3 and cerebral oedema. *Br Med J* 295:1486, 1987
9. BÜSING M, MELLERT J, GREGER B, HOPT UT: Acute pulmonary insufficiency due to OKT3 therapy. *Transplant Proc* 22:1779–1781, 1990
10. ROTHSTEIN JL, LINT TF, SCHREIBER H: Tumor necrosis factor/cachectin. Induction of hemorrhagic necrosis in normal tissue requires the fifth component of complement. *J Exp Med* 168:2007–2021, 1988
11. STEPHENS KE, ISHIZAKA A, LARRICK JW, RAFFIN TA: Tumor necrosis factor causes increased pulmonary permeability and edema. Comparison to septic acute lung injury. *Am Rev Respir Dis* 137:1364–1370, 1988
12. RAASVELD MHM, SURACHNO S, HACK CE, TEN BERGE RJM: Thromboembolic complications and dose of monoclonal OKT3 antibody. *Lancet* 339:1363–1364, 1992
13. RAASVELD MHM, HACK CE, TEN BERGE RJM: Activation of coagulation and fibrinolysis following OKT3 administration to renal transplant recipients: Association with distinct mediators. *Thromb Heamost* 68:264–267, 1992
14. HOHN DC, MEYERS AJ, GHERINI ST, BECKMANN A, MARKISON RE, CHURG AM: Production of acute pulmonary injury by leukocytes and activated complement. *Surgery* 88:48–58, 1980
15. STEVENS JH, O'HANLEY P, SHAPIRO JM, MIHM FG, SATOH PS, COLLINS JA: Effects of anti-C5a antibodies on the adult respiratory distress syndrome in septic primates. *J Clin Invest* 77:1812–1816, 1986
16. HOSEA SF, BROWN E, HAMMER C, FRANK MM: Role of complement activation in a model of adult respiratory distress syndrome. *J Clin Invest* 66:375–382, 1980
17. WARD PA, TILL GO, HATHERILL JR, ANNESLEY TM, KUNKEL RG: Systemic complement activation, lung injury, and products of lipid peroxidation. *J Clin Invest* 76:517–527, 1985
18. ROBBINS RA, RUSS WD, RASMUSSEN JK, CLAYTON MM: Activation of the complement system in the adult respiratory distress syndrome. *Am Rev Respir Dis* 135:651–658, 1987
19. MONTZ H, KOCH KC, ZIERZ R, GOTZE O: The role of C5a in interleukin-6 production induced by lipopolysaccharide or interleukin-1. *Immunology* 74:373–379, 1991
20. GOODMAN MG, CHENOWETH DE, WEIGLE WO: Induction of interleukin-1 secretion and enhancement of humeral immunity by binding of human C5a to macrophage surface C5a receptors. *J Exp Med* 156:912–917, 1982
21. OKUSAWA S, YANCEY KB, VAN DER MEER JWM, ENDRES S, LONNEMANN G, HEFTER K, FRANK MM: C5a stimulates secretion of tumor necrosis factor from human mononuclear cells *in vitro*. Comparison with secretion of interleukin-1 beta and interleukin-1-α. *J Exp Med* 168:443–448, 1988
22. HACK CE, HANNEMA AJ, EERENBERG AJM, OUT TA, AALBERSE RC: A C1-inhibitor-complex assay (INCA): A method to detect C1 activation *in vitro* and *in vivo*. *J Immunol* 127:1450–1453, 1981
23. HACK CE, PAARDEKOPER J, EERENBERG AJM, NAVIS GO, NIJSTEN MWM, THIJS LG, NUIJENS JH: A modified competitive inhibition radioimmunoassay for the detection of C3a. Use of ^{125}I-C3 instead of ^{125}I-C3a. *J Immunol Meth* 107:77–84, 1988
24. WOLBINK GJ, BOLLEN M, PAARDEKOOPER J, HACK CE: A sandwich enzyme-linked immunosorbent assay for the quantification of C4 activation products as a method to study complement activation via the classical pathway. (abstract) *Immunobiology* 184:459, 1992
25. NUIJENS JH, ABBINK JJ, WACHTFOGEL YT, COLMAN RW, EERENBERG AJM, DORS D, KAMP AJM, STRACK VAN SCHIJNDEL RJM, THIJS LG, HACK CE: Plasma elastase-α1-antitrypsin and lactoferrin in sepsis: Evidence for neutrophils as mediators in fatal sepsis. *J Lab Clin Med* (in press)
26. RAASVELD MHM, BLOEMENA E, WILMINK JM, SURACHNO S, SCHELLEKENS PTHA, TEN BERGE RJM: Interleukin-6 and neopterin in renal transplant recipients: A longitudinal study. *Transpl Int* (in press)
27. HACK CE, HART M, STRACK VAN SCHIJNDEL RJM, EERENBERG AJM, NUIJENS MWM, THIJS LG, AARDEN LA: Interleukin-8 in sepsis: Relation to shock and inflammatory mediators. *Infect Immun* (in press)
28. RODDIE ME, PETERS AM, DANPURE HJ, OSMAN S, HENDERSON BL, LAVENDER JP, CARROLL MJ, NEIRINCKX RD, KELLY JD: Inflammation: imaging with Tc-99m HMPAO-labeled leukocytes. *Radiology* 166:767–772, 1988
29. TODD PA, BROGDEN RN: Muromonab CD3. A review of its pharmacology and therapeutic potential. *Drugs* 37:871–899, 1989
30. HACK CE, PAARDEKOOPER J, SMEENK RJT, ABBINK J, EERENBERG AJM, NUIJENS JH: Disruption of the internal thioester bond in the third component of complement (C3) results in the exposure of neodeterminants also present on activation products of C3. An analysis with monoclonal antibodies. *J Immunol* 141:1602–1609, 1988
31. VERHOEVEN AJ, VAN SCHAIK MHJ, ROOS D, WEENING RS: Detection of carriers of the autosomal form of chronic granulomatous disease. *Blood* 71:505–507, 1988
32. KUYPERS TW, KOENDERMAN L, WEENING RS, VERHOEVEN AJ, ROOS D: Continuous cell activation is necessary for stable interaction of complement receptor type 3 with its counter-structure in the aggregation response of human neutrophils. *Eur J Immunol* 20:501–508, 1990
33. BORMER OP: Interference of complement with the binding of carcino-embryonic antigen to solid-phase monoclonal antibodies. *J Immunol Meth* 121:85–93, 1989
34. WAID TH, LUCAS BA, AMLOT P, JANOSSY G, YACOUB M, CAMMISULI S, JEZEK D, RHOADES J, BROWN S, THOMPSON JS: T10B9.1A-31 Anti-T-cell monoclonal antibody: Preclinical studies

and clinical treatment of solid organ allograft rejection. *Am J Kidney Dis* 14(Suppl 2):61–70, 1989
35. WAUWE J VAN, GOOSSENS J: Monoclonal anti-human T lymphocyte antibodies: Enumeration and characterization of T cell subsets. *Immunology* 42:157–164, 1981
36. CLARK M, COBBOLD S, HALE G, WALDMANN H: Advantages of rat monoclonal antibodies. *Immunol Today* 4:100–101, 1983
37. FERRAN C, DY M, MERITE S, SHEEMAN K, SCHREIBER R, LEBOULENGER F, LANDAIS P, BLUESTONE J, BACH JF, CHATENOUD L: Reduction of morbidity and cytokine release in anti-CD3 Mo-Ab treated mice by corticosteroids. *Transplantation* 50:642–648, 1990
38. CHATENOUD L, FERRAN C, LEGENDRE C, THOUARD I, MERITE S, REUTER A, GEVAERT Y, KREIS H, FRANCHIMONT P, BACH JF: *In vivo* cell activation following OKT3 administration; systemic cytokine release and modulation by corticosteroids. *Transplantation* 49:697–702, 1990
39. ZLABINGER GJ, STUHLMEIER KM, EHER R, SCHMALDIENST S, KLAUSER R, VYCHYTIL A, WATSCHINGER B, TRAINDL O, KOVARIK J, POHANKA E: Cytokine release and dynamics of leukocyte populations after CD3/TCR monoclonal antibody treatment. *J Clin Immunol* 12:170–177, 1992
40. CHATENOUD L, FERRAN C, BACH JF: The anti-CD3 induced syndrome: A consequence of massive *in vivo* cell activation. *Curr Top Microbiol Immunol* 174:121–134, 1991
41. TONNESEN MG, SMEDLY LA, HENSON PM: Neutrophil-endothelial cell interactions. Modulation of neutrophil adhesiveness induced by complement fragments C5a and C5a-DesArg and formyl-methionylleucyl-phenylalanine *in vitro*. *J Clin Invest* 74:1581–1592, 1984
42. LO SK, DETMERS PA, LEVIN SM, WRIGHT SD: Transient adhesion of neutrophils to endothelium. *J Exp Med* 169:1779–1793, 1989
43. WEISSMANN G, SMOLEN JE, KORCHAK HM: Release of inflammatory mediators from stimulated neutrophils. *N Engl J Med* 303:27–34, 1980
44. WEISS SJ: Tissue destruction by neutrophils. *N Engl J Med* 320:365–376, 1989
45. KOIVURANTA-VAARA P, BANDA D, GOLDSTEIN IM: Bacterial lipopolysaccharide induced release of lactoferrin from human polymorphonuclear leukocytes: Role of monocyte derived tumor necrosis factor α. *Infect Immun* 55:2956–2961, 1987
46. RICHTER J, ANDERSSON T, OLSSON I: Effects of tumor necrosis factor and granulocyte/macrophage colony stimulating factor on neutrophil degranulation. *J Immunol* 142:3199–3203, 1989
47. BAGGIOLINI M, WALZ A, KUNKEL SL: Neutrophil activating peptide/interleukin 8, a novel cytokine that activates neutrophils. *J Clin Invest* 84:1045–1049, 1989
48. MARTICH GD, DANNER RL, CESKA M, SUFFREDINI AF: Detection of interleukin-8 and tumor necrosis factor after intravenous endotoxin: The effect of inflammatory agents. *J Exp Med* 173:1021–1024, 1991
49. DEBETS JM, VAN DER LINDEN CJ, DIETEREN IE, LEEUWENBERG JF, BUURMAN WA: Fc-receptor cross linking induces rapid secretion of tumor necrosis factor (cachectin) by human peripheral blood monocytes. *J Immunol* 141:1197–1201, 1988
50. LING Z-D, ZILTENER HJ, WEBB BT, MATHESON DS: Aggregated immunoglobulin and Fc fragment of IgG induce IL-6 release from human monocytes. *Cell Immunol* 129:95–103, 1990
51. VAN DER POLL T, VAN DEVENTER SJH, HACK CE, WOLBINK GJ, AARDEN LA, BÜLLER HR, TEN CATE JW: Effects on the cytokine network, leukocytes and the complement system following injection of tumor necrosis factor into healthy humans. *Blood* 79:693–698, 1992
52. VAN DEVENTER SJH, HACK CE, WOLBINK GJ, VOERMANS HJ, STRACK VAN SCHIJNDEL RJM, TEN CATE JW, THIJS LG: Endotoxin-induced neutrophil activation. The role of complement revisited, in *Bacterial Endotoxins: Cytokine Mediators and New Therapies for Sepsis*, edited by LEVIN J, STURK A, TEN CATE JW, New York, John Wiley & Sons, Inc, 1991, p. 101