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Toxicity of OKT3 increases with dosage: a controlled study in renal transplant recipients

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Abstract In the present study we prospectively compared side effects occurring in 12 patients after the first administration of low-dose OKT3 (0.5 mg twice daily) induction therapy with those in 10 patients who were treated with a conventional dose of OKT3 (5 mg daily) for acute rejection. We also investigated cytokine release and activation of complement and neutrophils as all of these are held responsible for OKT3-induced side effects. Low-dose OKT3 resulted in a significantly decreased side effects score compared to that after a conventional dose of OKT3 (1.8 vs 5.1, $p = 0.0006$). Following the first administration of low-dose OKT3, TNF peak levels were significantly lower than after a conventional dose of OKT3. In contrast to our data on conventional dose OKT3 treatment, the first administration of low-dose OKT3 did not induce complement activation as reflected by C3a and C4b/c levels in plasma. Finally, the increase in neutrophil degranulation products lactoferrin and elastase-α_1-antitrypsin was much less following 0.5 mg OKT3 than following 5 mg. We conclude that OKT3-induced toxicity is dose-dependent and is mediated not only by cytokine release but also by activation of complement and neutrophils.

Key words OKT3, renal transplantation. Renal transplantation, OKT3 · Toxicity, OKT3, renal transplantation

Introduction

OKT3 is a murine monoclonal antibody (mAb) directed against the CD3 molecule on human T lymphocytes. Over the last decade it has been used both for prophylaxis and treatment of acute rejection episodes in organ transplant recipients [16, 23]. A first administration of OKT3 almost always causes clinical side effects, including pyrexia, dyspnea, headache, vomiting, and diarrhea [15, 22, 24]. Such side effects have been largely attributed to the release of cytokines as a result of transient activation of lymphocytes and monocytes by OKT3. Indeed, within hours after the first administration of OKT3 to renal transplant recipients, elevated levels of interleukin-2 (IL-2), interleukin-6 (IL-6), tumor necrosis factor (TNF), and gamma interferon (gamma IFN) are demonstrable that parallel clinical symptoms [1–4, 7, 8]. We have previously demonstrated that complement activation via the classical pathway occurs in renal transplant recipients treated with 5 mg OKT3 daily (conventional dose) [18]. This finding led us to suppose that complement activation and subsequent activation and degranulation of neutrophil granulocytes contribute to the side effects that occur following the first administration of a conventional dose of OKT3.

Recently, we conducted a controlled study in 50 renal transplant recipients comparing low-dose OKT3 prophylaxis (0.5 mg twice daily for 10 days) with prednisolone/cyclosporin therapy. The outcome of clinical parameters such as patient and graft survival, incidence of acute rejection episodes, and tolerance has been described in detail (Parlevliet, submitted): low-dose OKT3 induction therapy significantly reduced the incidence of acute rejections as compared to the usual immunosuppression with prednisolone and cyclosporin (21 % vs 52 %, $p = 0.02$). Furthermore, when compared to a historical control group of renal transplant patients in whom acute rejection was treated with a conventional dose of OKT3, low-dose OKT3 appeared to cause fewer side effects.

In the present paper, side effects following the first administration of low-dose OKT3 induction treatment in the first 12 out of 24 patients are prospectively compared to those in 10 patients who were treated with a conventional dose of OKT3 for acute rejection. This was done using a semiquantitative side effects score. We also report on both cytokine release and activation of complement and neutrophils, and we discuss the relationship between these parameters and the occurrence of clinical side effects.

Patients and methods

Patients and immunosuppressive protocols

Low-dose OKT3 study group

In a prospective study performed in our department from November 1990 through December 1991, 24 renal transplant recipients were prophylactically treated with low-dose OKT3 and compared to renal transplant patients who were treated with prednisolone/cyclosporin. Twelve low-dose OKT3-treated patients were monitored closely with respect to side effects, cytokine release, activation of the complement system, and neutrophil granulocytes. The low-dose OKT3 induction scheme was as follows: patients received azathioprine, 2.5 mg/kg per day, orally (the first dose was given preoperatively) and 50 mg prednisolone i.v. twice daily from the day of transplantation until the start of low-dose OKT3. In order to be able to monitor OKT3-induced side effects and immunological parameters, low-dose OKT3 prophylaxis (given twice daily as an i.v. bolus of 0.5 mg for 10 days) was started on the 3rd or 4th day after transplantation. Overhydration was corrected before the first administration of OKT3. One hour before the first administration of OKT3, 500 mg methylprednisolone (MPNS) was given as a single i.v. bolus, together with 25 mg promethazine orally. During OKT3 prophylaxis, azathioprine was continued as before, together with 25 mg prednisolone orally. Two days before the end of the OKT3 course, azathioprine was converted to cyclosporin, provided creatinine clearance was at least 30 ml/min. Prednisolone was gradually tapered off to 10 mg daily.

Conventional dose OKT3 study group

Ten renal transplant patients were treated for acute rejection with 5 mg OKT3, once daily, given as an i.v. bolus. Precautions taken, i.e., the dose of methylprednisolone and promethazine given and the time scale of drug administration, were identical to those of the low-dose group. Basic immunosuppression consisted of prednisolone/cyclosporin.

The study was approved by the ethics committee of the AMC and was performed according to the guidelines established in the 1964 Declaration of Helsinki. All patients gave their informed consent prior to their inclusion in the study.

Monitoring of side effects

In 12 low-dose and 10 conventional dose OKT3-treated patients, side effects were monitored closely and compared prospectively. We confined our study to the analysis of side effects occurring during the first 6 h following the first administration of OKT3. These data were noted and quantitated by two investigators (K.J.P. and F.J.B.) in the following way: from 0 to 3 h and from 3 to 6 h after OKT3 administration, patients were observed and questioned for the presence of (1) fever above 38.5 °C, (2) chills, (3) dyspnea, (4) nausea or vomiting, (5) diarrhea, and (6) headache. Each positive symptom scored 1+ when present during one of the intervals or 2+ when present during both intervals. Thus, the cumulative side effects score during the first 6 h following the first OKT3 administration ranged from 0 to 12.

Blood sampling and assays

Venous blood samples were obtained with the use of an indwelling catheter that was inserted into one of the femoral veins 1 h before the first administration of OKT3. According to the literature, time points for determination of cytokines, activated complement factors, and neutrophil degranulation products were chosen so as to result in an optimal reflection of kinetics [1, 3, 4, 18]. Blood samples were drawn just before and 30 min, 1 h, 3 h, and 6 h after the first three administrations of OKT3. Plasma TNF levels were measured with a commercially available ELISA (Medgenix, Billerica, Mass.). Plasma levels of complement activation products C3a-desarg and C4b/c were determined as previously described [9, 26]. Plasma levels of neutrophil degranulation products lactoferrin and elastase-α_1-antitrypsin (AT) complexes were measured with a RIA that has been described elsewhere [14]. The upper limit of values in plasma of 20 healthy controls, defined as mean +2 SD, was 40 pg/ml (TNF), 5 nmol/l (C3a-desarg) 55 nmol/l (C4b/c), 400 ng/ml (lactoferrin), and 100 ng/ml (elastase-α_1-AT).

In vivo complement fixation on lymphocytes by OKT3

In two patients the ability of OKT3 to bind C3b on T cells was determined as follows: before and 5, 15, 30, and 60 min after the first administration of 0.5 mg OKT3, heparin blood was collected and a suspension of peripheral blood mononuclear cells (PBMC) was obtained by means of density centrifugation (Ficoll Hypaque). PBMC were then incubated with a biotinylated mAb directed against a neoepitope on C3b, C3bi, and C3c (anti-C3-28) [10], followed by incubation with streptavidine-PE and double color stained with CD3-FITC (Leu-4, Beckton Dickinson, Mountain View, Calif., USA). Analysis was performed in the FACS (Becton and Dickinson, Mountain View, Calif., USA).

Table 1 Side effects score after first dose of OKT3. Numbers given indicate number of patients with complaints

	Time after start of OKT3 (hours)	Low-dose OKT3 (0.5 mg) $n = 12$	High-dose OKT3 (5 mg) $n = 10$
Dyspnea	0–3	1	4
	3–6	0	0
Chills	0–3	9	10
	3–6	1	1
Fever > 38.5 °C	0–3	2	9
	3–6	1	7
Headache	0–3	3	5
	3–6	2	5
Diarrhea	0–3	0	1
	3–6	1	1
Nausea/vomiting	0–3	1	4
	3–6	1	4
Total		22	51
Mean score		1.8	5.1

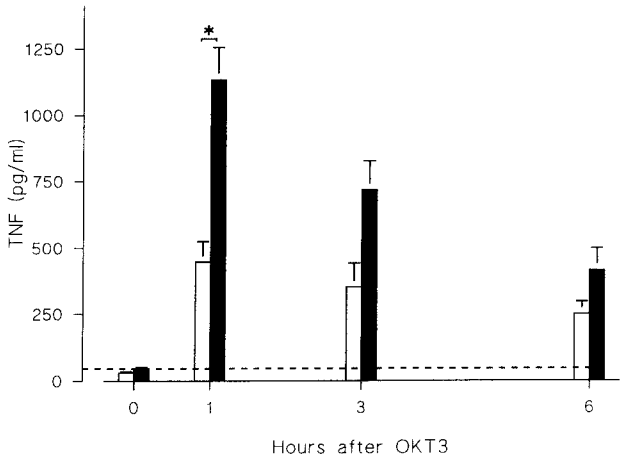

Fig. 1 Increase in TNF levels for 6 h after the first administration of 0.5 mg OKT3 (□, $n = 12$) and 5 mg OKT3 (■, $n = 10$). Results are shown as mean ± SEM. Time points shown are: immediately before and 30 min, 1 h, 3 h, and 6 h after OKT3 administration. The dotted line indicates upper limit of normal values. $P < 0.05$ (Mann-Whitney U-test)

Statistical analysis

Values are expressed as mean ± SEM. In each group of patients, peak levels of TNF, complement activation and neutrophil degranulation products were compared to pretreatment levels with the Wilcoxon signed rank test. Differences between the two groups were analyzed with the Mann-Whitney U-test. A p value below 0.05 was considered to be significant.

Results

Side effects after the first administration of 0.5 mg OKT3 were usually mild and subsided within a few hours (Table 1). Within minutes after the first administration, one patient complained of dyspnea that lasted for a few minutes. One hour after the first administration of 0.5 mg OKT3, 10 of the 12 patients experienced chills and/or fever. Gastrointestinal symptoms occurred in five patients and three patients complained of headache. In contrast, patients treated with 5 mg OKT3 experienced considerably more discomfort, not only on the 1st day of treatment but also on the 2nd and 3rd days. The relative distribution of side effects was similar in both groups. As a consequence, side effects scores after 0.5 mg OKT3 (average 1.8, range 0–4) were significantly lower than after 5 mg OKT3 (average 5.1, range 3–8, $p = 0.0006$, Mann-Whitney U-test).

Mean plasma levels of TNF are depicted in Fig. 1. Increases were only noted during the first 6 h following the first OKT3 administration. TNF levels increased significantly ($p < 0.01$, Wilcoxon signed rank test) after the first administration of OKT3 in both dosages, with peak values occurring at 1 h. TNF peak levels averaged 448 ± 78 pg/ml following 0.5 mg OKT3, while the administration of 5 mg OKT3 resulted in significantly higher peak levels (1133 ± 120 pg/ml, $p < 0.002$, Mann-Whitney U-test).

Although administration of 5 mg OKT3 resulted in significantly ($p < 0.05$, Wilcoxon signed rank test) increased peak levels of C3a and C4b/c (22.9 ± 2.8 and 119 ± 12 nmol/l, respectively) [18], plasma levels of C3a-desarg and C4b/c did not change after low-dose OKT3 administration (results not shown). However, in the two patients in whom this was tested in vivo, C3b fixation could be demonstrated on T lymphocytes 5 min after the first administration of 0.5 mg OKT3; however, 15 min after administration of the drug, this was no longer observed (data not shown).

In Fig. 2, levels of neutrophil degranulation products lactoferrin (Fig. 2A) and elastase-α_1-AT (Fig. 2B) are

depicted. Increases were only noted during the first 6 h of OKT3 administration. In the 5 mg OKT3 group, lactoferrin and elastase-α_1-AT levels were significantly increased ($p < 0.05$, Wilcoxon signed rank test) from 3 h and 0.5 h respectively to 6 h after the first OKT3 administration. In the 0.5 mg OKT3 group, significant increases compared to baseline values were only observed at 6 h, and the increase in lactoferrin levels remained within the normal range. In the 5 mg OKT3 group, lactoferrin peak levels of 815 ± 110 ng/ml were reached at 3 h. At this time, levels were significantly higher ($p < 0.05$, Mann-Whitney U-test) than those in the 0.5 mg OKT3 group. In the 0.5 mg group, average peak levels of lactoferrin were reached at 6 h, measuring 342 ± 72 ng/ml. Also, in the 5 mg OKT3 group, levels of elastase-α_1-AT were at first significantly ($p < 0.05$, Wilcoxon signed rank test) increased at 0.5 h, whereas in the 0.5 mg OKT3 group, this happened only after 6 h. Average peak levels of elastase-α_1-AT were slightly higher after 5 mg OKT3 (302 ± 42) than after 0.5 mg OKT3 (283 ± 96). Baseline values of elastase-α_1-AT levels were slightly elevated (162 ± 30 ng/ml) in 0.5 mg OKT3-treated patients.

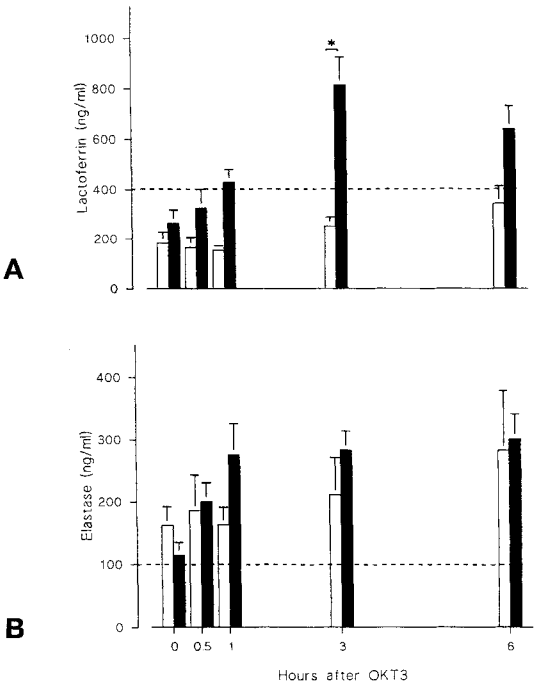

Fig. 2 A, B Neutrophil degranulation products **A** lactoferrin and **B** elastase-α_1-AT during 0.5 mg (□) and 5 mg (■) OKT3 treatment

Discussion

The present prospective study clearly indicates that the first administration of low-dose OKT3 causes significantly fewer side effects than does a conventional dose of OKT3. To our knowledge, the majority of studies on OKT3-induced side effects do not quantitate side effects or, if they do, they fail to describe how they quantitate them. Only recently, Gaston et al. [8] quantitated the severity of OKT3-related side effects by means of a reaction score. Since, in our experience, it is difficult to assess objectively the severity of side effects in individual patients, we used a semiquantitative side effects score derived at by adding up side effects during two separate intervals.

Current research on the side effects of OKT3 treatment is focused on the pathogenetic role of cytokines [1–4, 7, 8]. Indeed, the greater morbidity in our patients exposed to 5 mg OKT3 appears to be related to a higher TNF peak level than in patients treated with 0.5 mg OKT3. Although basic immunosuppression was not the same in the two groups of patients, it seems unlikely that out results can be attributed to these differences. First, the dose of prednisolone in both groups was comparable, and second, the group with the more severe side effects received cyclosporin rather than azathioprine, a drug known for its better suppression of T-cell reactivity. Nevertheless, one cannot rule out the possibility that the presence of activated T cells and their interaction with OKT3 in patients with a rejection contributed to the increased number of side effects. Yet, cytokines may not be the only mechanism responsible for OKT3-induced side effects. Recently, we reported that within 15 min after the first administration of 5 mg OKT3, complement activation via the classical pathway occurred, followed by early activation of neutrophils [18]. The late and sustained activation of neutrophils contributed to the release of cytokines [5, 13, 17, 19]. As with septic shock, activation of complement and neutrophils has been considered a possible pathogenetic factor in the development of side effects following the first administration of OKT3 [11, 12, 14, 20, 21, 25].

In the present study, no circulating complement activation products were observed at any time point following the first administration of low-dose OKT3. Also, an early increase in neutrophil activation products was completely absent in the 0,5 mg OKT3 group. These results support the hypothesis that the early increase in neutrophil degranulation products following the first administration of 5 mg OKT3 is induced by systemic activation of complement. Although the first administration of low-dose OKT3 leads to complement fixation on T cells, this apparently is insufficient to result in detectable plasma levels of complement activation products. The late increase in neutrophil degranulation products after administration of 0.5 mg OKT3 can be attributed to the release of cytokines. Finally, the slightly elevated baseline levels of elastase-α_1-AT in the 0.5 mg

OKT3 induction group can be attributed to recent surgery (i.e., kidney transplantation) [6]. This maker the comparison with the high-dose group somewhat difficult, although it does not alter the kinetics of the elastase response.

In conclusion, our data clearly indicate that low-dose OKT3 treatment causes less morbidity than conventional dose OKT3 treatment. In addition, our results indicate that both cytokine release and activation of complement and neutrophils after OKT3 administration are dose-dependent and contribute to OKT3-related toxicity. Since OKT3 is an effective immunosuppressive agent, even at a low dose, we stress the need to conduct proper dose-finding studies on OKT3 in order to develop protocols in which adequate immunosuppression is combined with the fewest possible side effects.

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