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Original article

Anti-OKT3 response following prophylactic treatment in paediatric kidney transplant recipients

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Abstract. The anti-OKT3 response was studied in 40 paediatric kidney transplant recipients receiving OTK3 as a prophylactic treatment in association with azathioprine and prednisone. Only 1 patient experienced a reversible acute rejection episode while receiving OKT3. OKT3 induced a rapid disappearance of CD3+ cells, but significant proportions of CD3+ cells reappeared before the end of the treatment in 14 patients. Wide variations in circulating OKT3 levels were observed and in only 50% of patients could stable circulating OKT3 levels be detected until discontinuation of treatment. Anti-OKT3 antibodies detected by the enzyme-linked immunosorbent assay (ELISA) (anti-idiotypic and anti-isotypic antibodies) developed in 91% of patients. Anti-idiotypic antibodies detected by the immunofluorescence inhibition test were found in the sera of 71% of patients, always when high titres of anti-OKT3 antibodies were detected by ELISA. As it has recently been shown that anti-idiotypic antibodies are associated with failure of subsequent OKT3 treatment, we conclude that OKT3 should be restricted to steroid-resistant rejection crises in paediatric patients.

Key words: OKT3 – Kidney transplantation – Anti-OKT3 antibodies – Immunosuppression – Monoclonal antibodies

Introduction

The monoclonal antibody OKT3 has been used extensively in organ transplantation for more than 10 years. OKT3 has been shown to be an effective therapy for acute renal, cardiac and liver allograft rejection [1–4]. A multicentre trial has shown that OKT3 is more effective than high doses of steroids in reversing acute rejection following cadaveric kidney transplantation [5]. OKT3 is also effective for steroid-resistant rejections [6, 7]. The OKT3 monoclonal antibody has also been used for the prevention of rejection, starting at the time of transplantation. The few published reports indicate that prophylactic OKT3 is effective in reducing the incidence of early acute rejection [8–11].

One of the important side effects of OKT3, which is common to all murine monoclonal antibodies, is the production by the recipient of antibodies directed against the xenogenic molecule [12]. Xenosensitization does not lead to the hypersensitivity reactions seen with polyclonal antilymphocyte sera. The main consequence is the accelerated clearance of the injected OKT3 which limits its therapeutic effectiveness [13, 14] and also precludes a second course of OKT3 treatment [15, 16]. The incidence of anti-OKT3 sensitization depends on the dose of immunosuppressive drugs used concomitantly. The concurrent administration of azathioprine and/or cyclosporine has been shown to be efective in lowering the incidence of sensitization as well as the anti-OKT3 antibody titres [9, 17, 18].

In children, the immunosuppressive effect of OKT3 appears to be similar to that described in adults [19]. However, the immune response of paediatric patients has not been studied as extensively as in adults. In this report, we describe our experience with OKT3 in the prophylactic treatment of children receiving kidney transplantation and the immunological monitoring of these patients.

Patients and methods

Patient population

From September 1987 to November 1989, 40 children (22 boys, 18 girls) who received 35 primary and 5 secondary cadaver kidney allografts were included in this study. The age of these patients ranged from 2 years 8 months to 18 years 5 months. The number of HLA-A, -B and -DR compatibilities varied from 2 to 5.

A sequential immunosuppressive treatment consisting of OKT3, azathioprine and prednisone followed by cyclosporine was used in these patients. IV OKT3 (0.1 mg/kg body weight per day) was administered intra-operatively and continued for 21 days in the first 18 patients

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(group A) and for 14 days in 22 patients (group B). Methylprednisolone (60 mg/m^2 IV) was administered before the first two injections of OKT3, followed by oral prednisone (60 mg/m^2 per day) in two doses, tapered to 30 mg/m^2 by day 30. Azathioprine was administered at a dose of 1.5 mg/kg body weight per day. Cyclosporine (150 mg/m^2 per day in two oral doses) was started 2 days before OKT3 withdrawal and the dose was adjusted to achieve trough whole blood levels of 100–200 ng/ml, as measured by a radioimmunoassay using monoclonal antibodies.

Acute tubular necrosis

Acute tubular necrosis was defined as a failure of serum creatinine to decrease in the absence of haemodialysis treatment. Acute tubular necrosis was considered to have resolved on the 1st day serum creatinine started to decrease without haemodialysis.

Monitoring of peripheral blood T cells

Peripheral blood T lymphocytes were monitored prior to the first injection and three times weekly thereafter. Peripheral blood mononuclear cells were isolated using standard techniques. T cells were evaluated by indirect immunofluorescence using the OKT3 monoclonal antibody and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG2a.

Evaluation of serum OKT3 levels

Serum OKT3 levels were serially monitored by a specific enzyme-linked immunosorbent assay (ELISA). The ELISA was performed using purified goat anti-mouse IgG-coated wells (2 µg/well). Test sera were diluted 1/100 and an appropriate dilution of goat anti-mouse IgG peroxidase conjugate was added after a 2-h incubation. After a further 2-h incubation, OKT3 binding was revealed using orthophenylenediamine as the peroxidase substrate and the optical density was read at 492 nm. The concentration of OKT3 in each sample was calculated by extrapolating the optical density value obtained from a reference curve obtained with serial dilutions of normal human serum containing purified OKT3.

Detection of anti-OKT3 antibodies

The presence of anti-OKT3 antibodies was assessed in parallel by two assays.

Anti-OKT3 ELISA. ELISA microplates were coated with pure OKT3 (0.2 µg/well). After incubation and washes, test sera (diluted 1/200) were added for 2 h. Following washes, the plates were incubated with peroxidase-labelled goat anti-human IgG or goat anti-human IgM antiserum and the binding of the peroxidase conjugate was revealed as described for the OKT3 ELISA. Results were expressed by the following index:

$$\text{This is a ratio: } \frac{\text{Mean absorbance of test sample}}{\text{Mean absorbance of negative control}}$$

A test sample was considered positive if the index was 1.8 or higher.

Inhibition of binding of OKT3 to normal T cells (immunofluorescence-inhibition assay). Appropriate concentrations of OKT3 were incubated with the test serum and normal peripheral blood mononuclear cells were added after 30 min. Following incubation, the cells were washed and labelled with FITC-conjugated goat anti-mouse IgG2a antiserum. The percentage of labelled cells was evaluated using an Ortho Cytofluorograph.

Results

Acute tubular necrosis

Acute tubular necrosis occurred in 33 of the 40 patients who all received cadaveric grafts. Except for 2 cases of primary allograft non-function, acute tubular necrosis resolved before day 7 in 15 patients, between day 7 and day 14 in 13 patients and between day 14 and day 30 in 10.

Acute rejection

Only 1 patient experienced an acute reversible rejection episode during the period of OKT3 therapy. This rejection episode was documented by fine needle aspiration cytology 11 days after transplantation. Ten patients (2 in group A and 8 in group B) developed an acute rejection episode during the 1st month following transplantation and 15 patients (9 in group A and 6 in group B) during the 2nd month or later after transplantation. Overall, 26 of 40 patients showed evidence of acute rejection. With a follow-up of 2 years 4 months to 4 years 6 months, 11 patients did not show any sign of rejection.

Patient and graft survival

One patient in group B died during the 2nd month after transplantation from cytomegalovirus (CMV) infection with pneumonitis and myocarditis, despite gancyclovir treatment. Seven kidneys were lost, 3 from vascular thrombosis, 1 from renal necrosis occurring after a collapse due to immediate bleeding and the remaining 3 from rejection 2 months to 2 years following transplantation. Vascular thrombosis occurred immediately after transplantation in 1 patient, following an operation for ureteral obstruction on day 14 in a second patient and after surgery for renal artery stenosis 2 years after transplantation in the third patient. Histological examination showed no sign of rejection in the 2 patients with early thrombosis.

Infectious complications

Of the 40 patients, 22 experienced infectious complications during the first 2 months. Bacterial infections were observed in 14 patients, including lower urinary tract infection in 12, salmonella infection in 1 and wound infection in 1. A clinically significant CMV infection occurred in 7 patients, 4 of whom developed pneumonitis. One of these patient died from CMV infection.

Peripheral CD3+ cells

In all patients, the dose of 0.1 mg OKT3/kg body weight induced a rapid and total depletion of CD3+ peripheral lymphocytes. CD3+ cells, as assessed by fluorescence microscopy, remained undetectable during effective OKT3

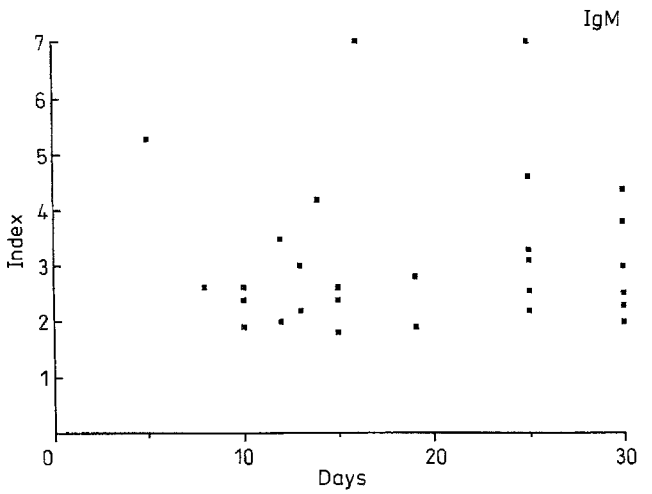

Fig. 1. Kinetics of sensitization in patients treated with OKT3. IgM antibodies

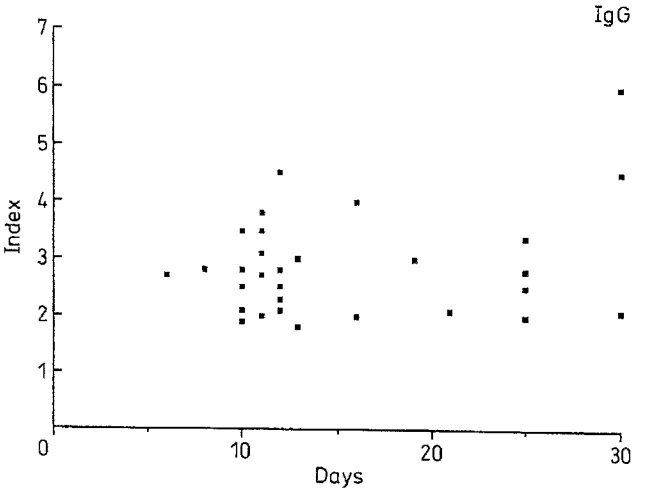

Fig. 2. Kinetics of sensitization in patients treated with OKT3. IgG antibodies

treatment. Circulating T cells coated with OKT3 could not be detected at any time during the treatment course.

In 9 patients of the 18 treated for 21 consecutive days, CD3+ cells remained absent from the circulation throughout the treatment period. In the remaining 9 patients, they reappeared in a significant proportion by 10–18 days. A similar pattern was observed among the patients treated for 14 consecutive days, as in 5 patients CD3+ cells reappeared by 11–14 days whereas they remained absent from the circulation in the remaining 14 patients. In all patients, the reappearance of fully labelled CD3+ cells in the circulation, either due to cessation of treatment or to premature xenosensitization, was rapid, reaching values ranging from 30% to 65% of peripheral blood mononuclear cells in 48–72 h.

Circulating OKT3 levels

As in OKT3-treated adult patients [20], the circulating mouse monoclonal antibody levels showed wide individual variations. Plateau levels were regularly reached by day 2–day 4 of consecutive treatment and ranged from 380 to 3,100 ng/ml. In the patients in whom stable OKT3 circulating levels were detected until discontinuation of therapy, CD3+ cells remained absent from the circulation. This occurred in 9 patients treated for 3 weeks (group A) and in 11 patients treated for 2 weeks (group B). Conversely, in 9 patients of group A and 11 patients of group B, premature disappearance of OKT3 trough levels could be demonstrated by 10–18 days of treatment. In 9 patients in group A and in 5 patients in group B, this disappearance of OKT3 trough levels correlated with a rapid reappearance of circulating CD3+ cells. In the remaining 5 patients in group B, OKT3 had disappeared from the circulation by day 11–13 and CD3+ cells reappeared 24–48 h thereafter.

Anti-OKT3 antibodies

The specificity of the response to OKT3 was assessed by using two different techniques in parallel, namely a specific ELISA and the immunofluorescence inhibition assay. The ELISA detects both anti-idiotypic antibodies and anti-isotypic antibodies. Using this assay, we found that the frequency of sensitization was 83% in group A and 95% in group B. These figures exclude 4 patients in group A and 1 patient in group B from whom no sera were available after the end of the treatment to check for the presence of anti-OKT3 antibodies. Figures 1 and 2 show the kinetics of sensitization, IgM and IgG anti-OKT3 antibodies appearing 5–30 days after initiation of therapy.

The immunofluorescence inhibition assay allows the detection of free anti-OKT3 antibodies which block the binding of OKT3 to its target cell antigen. These anti-OKT3 antibodies are anti-idiotypic antibodies. Using the immunofluorescence inhibition test, two sorts of patterns could be shown in the 34 patients studied: a total inhibition in 16 patients (47%) and a partial inhibition in 8 patients (23.5%). These data underline the sensitivity of the detection. In all cases, a positive immunofluorescence inhibition test correlated with very high anti-OKT3 antibody levels as detected by the ELISA.

In 16 patients, a positive ELISA was observed during the treatment course while the immunofluorescence inhibition test was negative and circulating OKT3 was detectable using the conventional ELISA. In all these patients, xenosensitization correlated with the accelerated disappearance of circulating OKT3 and paralleled the premature reappearance of CD3+ circulating cells. The pattern exhibited by 1 representative patient is shown in Fig. 3.

After the end of the treatment, a positive ELISA result was observed in 7 patients while the immunofluorescence inhibition assay was negative. This may be related to an exclusive or nearly exclusive anti-isotypic antibody response in these patients.

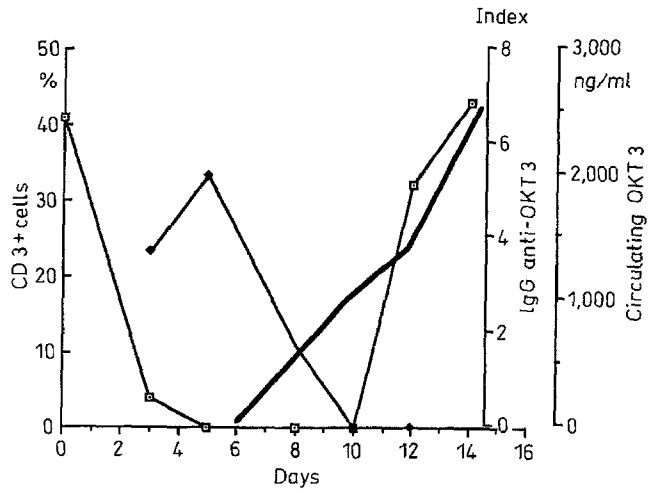

Fig. 3. Representative pattern of anti-OKT3 immunization in a patient. ———, IgG anti-OKT3; □, CD3 + cells; ■, circulating OKT3

Discussion

The OKT3 monoclonal antibody was used in the prophylactic treatment of 40 children who received a cadaveric kidney transplant. OKT3 given together with azathioprine and steroids was effective, as only 1 patients developed an acute rejection while receiving OKT3. Following completion of OKT3 therapy, 10 patients experienced an acute rejection episode during the 1st month after transplantation and 15 others later. The incidence of rejection episodes after OKT3 therapy was similar to that of a group of patients receiving anti-lymphocyte serum instead of OKT3 as a prophylactic treatment [21]. OKT3 treatment was not associated with an increase in infectious complications compared with the group of patients receiving anti-lymphocyte serum.

A careful monitoring of CD3+ cells showed that significant proportions reappeared in the circulation after 10 days of treatment in 14 patients. This was correlated with a rapid disappearance of circulating OKT3 antibody. This study shows that the rapid decrease in circulating mouse monoclonal antibodies was due to formation of an anti-OKT3 antibody. Anti-OKT3 antibody production was studied during and following OKT3 therapy. The overall frequency of anti-OKT3 antibody sensitization was close to 90%, as assessed by the ELISA which detects both anti-isotypic and anti-idiotypic antibodies. The immunofluorescence inhibition assay, which is specific for anti-idiotypic antibodies, was positive in 70% of patients. These anti-idiotypic antibodies, unlike anti-isotypic antibodies, neutralize the in vivo immunosuppressive activity of OKT3 by interfering with its binding site. Therefore, the early production of anti-idiotypic antibodies during the treatment course was responsible for the rapid reappearance of CD3+ cells.

During the treatment course, some patients showed a positive ELISA result without anti-idiotypic antibodies. This may be related to a very early detection of free anti-OKT3 antibodies or of circulating OKT3/anti-OKT3 complexes appearing at the beginning of the immunization process. However, it must be stressed that the ELISA used for the detection of circulating OKT3 does not allow reliable distinction between free OKT3 and OKT3/anti-OKT3 complexes. Nevertheless, at least part of the detected OKT3 must have been circulating free, with a combining site, since CD3+ cells were absent from the circulation.

The incidence of anti-OKT3 antibody production following OKT3 therapy has been studied by several authors [12–14, 18, 21]. When OKT3 was used alone for prophylactic treatment after kidney transplantation, almost all patients developed anti-OKT3 antibodies within 10 days [9, 11, 13]. The association of conventional immunosuppressive therapy with OKT3 has been shown to decrease the incidence of anti-OKT3 sensitization. Only 50% of patients receiving azathioprine and low-dose prednisolone develop anti-OKT3 antibodies [9]. Ciclosporine has also been found to prevent the anti-OKT3 response [17], but the combination of these two agents is associated with an increased risk of lymphoproliferative syndromes.

In paediatric renal transplant recipients receiving OKT3 for prophylaxis or rejection, Schroeder et al. [22] reported antibody formation rates of 45%. These patients were also receiving azathioprine and prednisone, while ciclosporine was begun 3 days before the end of therapy. Leone et al. [19] reported antibody formation rates of 85% in paediatric renal transplant recipients. Ciclosporine was discontinued and reinstituted 4 days before the end of treatment.

Legendre et al. [23] recently reported that the presence of anti-idiotypic antibodies detected by the immunofluorescence-inhibition assay was associated with a failure of OKT3 retreatment. Conversely, those patients with no evidence of anti-idiotypic antibodies could be successfully retreated with OKT3, irrespective of the anti-OKT3 antibody titres detected by ELISA. Therefore, our data suggest that a second course of OKT3 will be ineffective in most children. Consequently, we suggest that OKT3 treatment should be restricted to steroid-resistant rejection crises and should not be used as a prophylactic treatment for rejection in paediatric patients.

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Literature abstracts

Pediatrics (1992) 89: 1072–1074

Renal tubular acidosis in children treated with trimethoprim-sulfamethoxazole during therapy for acute lymphoid leukemia

Jerome Linus Murphy

The antibiotics trimethoprim (TMP) and sulfamethoxazole (SMZ), when used in combination, can cause metabolic acidosis, renal bicarbonate wasting, and growth failure. Retrospective review of repeated random serum chemistries from 10 children receiving TMP-SMZ and maintenance chemotherapy for acute lymphoid leukemia revealed low serum bicarbonate ($P = .0002$) and elevated serum chloride ($P < .0005$) concentrations. These values normalized after all medications were discontinued. Prospective study of 8 children receiving TMP-SMZ and chemotherapy for acute lymphoid leukemia revealed lower serum bicarbonate concentrations and higher urine pH following a dose of TMP-SMZ than paired values obtained more than 3 days after a dose. Four children (50%) met serum bicarbonate and urinary pH criteria for the diagnosis of renal tubular acidosis soon after a dose of TMP-SMZ. The occurrence of TMP-SMZ-induced renal tubular acidosis has implications for the acid-base balance of children receiving TMP-SMZ on a long-term basis.

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Role of a streptococcal antigen in the pathogenesis of acute poststreptococcal glomerulonephritis. Characterization of the antigen and a proposed mechanism for the disease

Nobuyuki Yoshizawa, Satoshi Oshima, Inge Sagel, Jun Shimizu, and Gerhard Treser

We studied the significance of a streptococcal protein (preabsorbing Ag) (PA-Ag) in the pathogenesis of acute poststreptococcal glomerulonephritis (APSGN). This protein was isolated from nephritogenic streptococci. Purification of PA-Ag was achieved by chromatography, followed by Sephadex IEF. A single protein band at pH 4.7 was identified as PA-Ag. The m. w. was 43,000. Rabbit antisera against PA-Ag and sera of patients with APSGN showed identical precipitation lines by immunodiffusion. Antibodies to PA-Ag were found to be pressent in 30 of 31 patients with APSGN, in 1 of 36 patients with uncomplicated group A streptococcal upper respiratory tract infections, and in 1 of 36 normal adults. By using immunoelectrophoresis, it was found that PA-Ag activates the alternate pathway of C. Other water-soluble streptococcal fractions, used as controls, did not activate the C system. The demonstration that PA-Ag is present in the glomeruli in the early phase of APSGN and its ability to activate C3 and factor B suggest that PA-Ag may be involved in the pathogenesis of APSGN, via in situ C activation.