

MONOCLONAL ANTIBODY THERAPY

ANTI-IDIOTYPIC AND NON-ANTI-IDIOTYPIC ANTIBODIES TO OKT3 ARISING DESPITE INTENSE IMMUNOSUPPRESSION¹

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The frequency, timing, and specificity of the humoral antibody response to a murine monoclonal antibody (OKT3, IgG_{2a}) were measured in 21 consecutive renal allograft recipients. These patients received i.v. OKT3, 1–5 mg/day for 10–20 days as treatment for acute graft rejection. Maintenance immunosuppression consisted of azathioprine and corticosteroids. Using three different assays, an antibody response was detected in 75% of the 20 patients with adequate samples. The ELISA assay of the overall IgM and IgG reactivity to OKT3 revealed that IgM anti-OKT3 appeared in 65% and IgG anti-OKT3 in 50% of the patients, reaching a peak 20–33 days after the last dose of OKT3. The IgM preceded the IgG in most cases ($P < 0.02$) and in 8 cases was detected during therapy. One patient had high levels of IgM anti-OKT3 before therapy, yet responded normally to OKT3. Interference with the therapeutic effectiveness was evident in one patient who developed IgG antibodies during therapy. His serum blocked the binding of F-OKT3 to normal lymphocytes in the presence of normal BALB/c serum. The blocking assay, done by flow cytometry, measured anti-idiotypic (Id) reactivity since the sera did not affect the binding of OKT8 (another IgG_{2a}) or anti-Leu4 (another anti-T3), and the blocking activity remained after affinity absorption with normal mouse IgG. Using this assay, 60% of the patients made an anti-Id response. One made only anti-Id, and several had anti-Id at times when other reactivities were undetectable. Antibodies to non-idiotypic, presumably isotypic, determinants represented on OKT8 occurred in only 44%, while other reactivity (OKT4; IgG_{2b}) was less common (12%) and weaker.

While no adverse allergic reactions occurred in this group of patients, the anti-Id antibodies, which are a prominent feature of the immune response to this and probably other monoclonal antibodies, can block their therapeutic effectiveness and can arise despite intense immunosuppression. This response may require the use of different idiotypes for prolonged or repeated courses of therapy and may be the major obstacle to the use of human monoclonal antibodies.

The parenteral administration of foreign serum proteins has been known since the early days of serotherapy to stimulate an immune response. The typical allergic manifestations include fever, skin rash, anaphylaxis, and serum sickness. This phe-

nomenon has acquired new relevance with the application of murine hybridoma-derived antibodies to clinical medicine. Despite some initial optimism, the injection of these murine proteins into man, as expected, regularly provokes an immune response by the host (1–11).

We have had extensive experience with the use of one monoclonal antibody, OKT3 (IgG_{2a}, BALB/c), used as an adjuvant to azathioprine and prednisone for treatment of acute renal allograft rejection (1, 2, 11). This antibody, reactive with an invariant component (T3)* of the T cell antigen receptor complex (12), has proved to be dramatically effective in reversing acute cellular rejection when administered at doses of 1–5 mg/day over 10–20 days. This report describes in detail the specificity of the antibody response to OKT3 in 21 renal allograft recipients, and the implications for therapy. A preliminary report of the first 11 patients has been presented (6, 7).

MATERIALS AND METHODS

Patients. The 21 consecutive OKT3-treated patients studied were initially treated with prednisone and azathioprine after receiving a cadaver donor renal allograft (1, 2). They were given OKT3 (Ortho Pharmaceuticals, Raritan, NJ) when they had their first episode of renal allograft rejection. The antibody was given once daily as a 1–5-mg i.v. bolus. No patient manifested a skin reaction to intradermal OKT3 (0.1 µg) prior to treatment. In an attempt to modify the immune response to OKT3, 6 patients also received cyclophosphamide (patients Nos. 10 and 17–21).

Serum samples. Blood samples were collected in acid-citrate-dextrose (ACD) at least twice weekly beginning at the time of transplantation and continuing for up to ten months after OKT3 therapy. An average of 19 samples per patient were analyzed in 20 patients (range 12–24). One patient (No. 3) had only 3 posttreatment samples (all negative) and therefore was not included in further analysis. The samples were centrifuged at 1500 rpm and the plasma was stored at –20°C. Peripheral blood lymphocytes from these samples were used for immunologic monitoring as described previously (1).

Enzyme-linked immunosorbent assay (ELISA). This assay, performed by a slight modification of published methods (13), was used to detect both anti-Id and non-anti-Id anti-OKT3 antibodies. Polyvinyl vinyl plastic microtiter plates (Imulon I,

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* Abbreviations used: ACD, acid citrate dextrose; CAF₁, (BALB/c × A/J)F₁; ELISA, enzyme-linked immunosorbent assay; F-, fluorescein-conjugated (e.g., F-OKT3); Id, idiotype; MFC, median fluorescence channel; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; T3, the 20–25 kilodalton surface antigen of mature T cells.

Dynatech, Alexandria, VA) were coated with 1 $\mu\text{g}/\text{ml}$ of purified parenteral grade OKT3, 0.05 M NaHCO_3 buffer (pH 9.6). The plates were incubated overnight at 4°C then washed with phosphate-buffered saline containing .05% (v/v) Tween-20 (0.01 M phosphate, 0.14 M NaCl, (pH 7.4) (PBS-Tween). Each serum sample was diluted 1:240 in PBS-Tween (5 $\mu\text{l}/1.2$ ml buffer). 200 μl of this mixture was added to the wells. The plates were incubated for 2 hr at room temperature, washed with PBS-Tween, and 200 μl of a 1:500 dilution of alkaline-phosphatase-conjugated goat antihuman IgG (Kirkegaard-Perry, Gaithersburg, MD)—or goat antihuman IgM (both heavy chain specific) was added at a similar concentration. Anti-IgG with light chain reactivity was used in early studies as a screening test. These solutions were incubated for 2 hr at room temperature and washed again using PBS-Tween. Finally, 200 μl of 1 mg/ml p-nitrophenyl phosphate in 10% diethanolamine was added. The resultant color change of substrate was read on an ELISA plate spectrophotometer (Bio-Tek Instruments) at 405 nM. Sera from 15 healthy volunteers and assays without OKT3, serum, or anti-Ig were used as controls. All assays were done in triplicate. Each daily run was standardized by running aliquots of a known positive control. This serum had a net OD_{405} (test-blank) of about 650 and 70 for the IgG and IgM assays, respectively. Standardized values for IgG were obtained by multiplying the net OD_{405} of the test sample by $650/(\text{OD}_{405}$ of standard) on that day (or $70/\text{OD}_{405}$ of standard for IgM). A sample was considered positive if the corrected OD_{405} was 2 SD above the mean of the controls (95th percentile) in the case of the pretreatment samples, or if the OD_{405} was more than double the pretreatment OD_{405} in the case of subsequent samples (provided the OD_{405} was >100).

PHA blast preparation. The preparation of phytohemagglutinin (PHA)-stimulated T cell blasts was accomplished by methods previously described (14). Briefly, normal peripheral mononuclear cells were isolated on Ficoll-Hypaque gradients and stimulated with 1 μg of PHA per 10^6 lymphocytes in 1 ml of RPMI 1640 medium containing 5% normal human serum, antibiotics, and 5 μM mercaptoethanol. Cells were maintained by dilution to $10^5/\text{ml}$ in T cell growth factor medium containing interleukin-2 about every third day.

Enhancing assay for non-Anti-Id antibodies. Using flow cytometry, non-anti-Id human antibodies that included isotype-specific reactivity to murine IgG_{2a} were detected by the binding to OKT8-coated ($\text{IgG}_{2a\kappa, \text{CAF}_1}$) PHA blasts. Antibodies not specific for Id or isotype were similarly measured with OKT4-coated ($\text{IgG}_{2b\kappa, \text{CAF}_1}$) targets. OKT3-coated targets were used as a positive control. PHA blasts (which contained both $\text{T3}^+\text{T4}^+$ and $\text{T3}^+\text{T8}^+$ cells) were suspended at a concentration of $10^6/\text{ml}$ in RPMI 1640. Nonfluoresceinated OKT8, OKT4, or OKT3 (0.5 μg) or PBS was added to each tube containing 50 μl of PHA blasts. The suspensions were incubated at 4°C for 30 min and washed twice with PBS. 50 μl of test or normal serum was added and the mixture incubated and washed as before. Fluoresceinated goat antihuman IgG (heavy and light chain reactive) was then added and the tubes were incubated and washed as before. The median intensity of the positive staining was evaluated using the Spectrum III flow cytometer (Ortho Diagnostics System, Westwood, MA) (15). The percentage of increase in the median fluorescence channel (MFC, linear units) above uncoated blasts (no monoclonal antibody) for each sample was calculated. This corrects for any contribution by anti-T-cell antibodies that may be present in the patient's serum

e.g., anti-HLA. Positive values were defined as 2 SD units above the mean of 21 normals.

Affinity columns. OKT3 and normal mouse IgG (Miles) were coupled to CNBr-activated Sepharose 4B (Pharmacia) by standard techniques, with approximately 10 mg of protein/ml swollen gel. Columns were 12×0.9 cm (mouse IgG) and 4.5×0.9 cm (OKT3). Fractions were eluted in PBS and then 3M KSCN, dialyzed, and reconcentrated to the starting volume by positive pressure filtration (Amicon XM-50).

Anti-idiotypic (Id) assay. The basis of this assay is that certain anti-Id block the binding of the Id-bearing antibody with antigen. The test was done by flow cytometry using fluoresceinated OKT3 (F-OKT3) as the Id and T3^+ T cell blasts (or normal peripheral blood lymphocytes) as the antigen in the presence of the test serum. Normal BALB/c mouse serum was added to block non-anti-Id antibodies. The controls included normal human serum and fluoresceinated OKT8 (F-OKT8). The amount of F-OKT3 required to saturate PHA blasts or peripheral lymphocytes was determined by titration with a constant number of cells, plotting the MFC-vs.-concentration of F-OKT3 used (Fig. 1). Saturation was achieved at 8 $\mu\text{g}/\text{ml}$ of F-OKT3; beyond this point no further shift in the MFC was achieved. A point of about 30% saturation was selected (2.5 $\mu\text{g}/\text{ml}$) for use in the assays to maximize sensitivity. A similar point was determined for F-OKT8.

The assay was performed by adding 25 μl of test or normal serum to 20 μl of BALB/c serum and incubating for 30 min at room temperature. Subsaturing amounts of F-OKT3 or F-OKT8 were added and these were incubated for 30 min on ice. Normal buffy coat peripheral blood lymphocytes (25 μl), previously prepared from ACD blood, were added to each tube with further incubation for 30 min at 4°C. Residual erythrocytes were lysed with NH_4Cl and the cells were washed twice in PBS. Samples were analyzed using the Spectrum III flow cytometer gated on the lymphocyte or lymphoblast cluster. The MFC of the positive cells above the positive threshold channel was taken as the index of staining intensity. Pretreatment samples had inhibition of $7 \pm 7\%$. Therefore a decrease in the MFC of greater than 20% of the normal serum control, provided there was not a concomitant inhibition of F-OKT8, was taken as evidence for the presence of anti-Id antibody. Samples

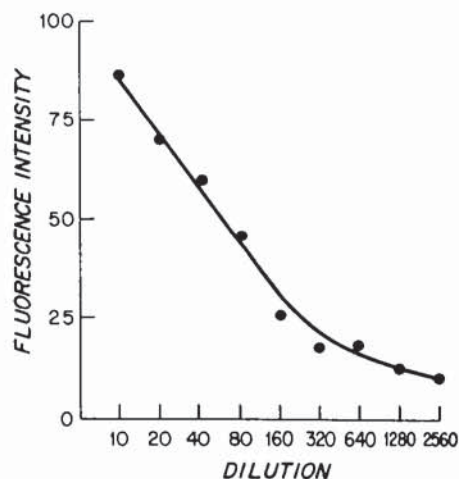


FIGURE 1. Plot of fluorescence intensity (MFC, linear units) vs. dilution of F-OKT3 on PHA lymphoblasts. A point of about 30% saturation was selected for the anti-Id inhibition assay.

containing no BALB/c serum were analyzed in parallel. F-anti-Leu 4 (Becton Dickinson Monoclonal Center, Mountain View, CA) was used for comparison.

Statistical analysis. Values given are mean \pm SD unless otherwise noted. Student's *t* and Fisher's exact tests were used for evaluating statistical significance. An antibody response was considered positive if two or more sample values were above the defined threshold, either a doubling of the pretreatment value or above the 95th percentile of normal controls.

RESULTS

ELISA: total Anti-OKT3 response. Using the ELISA with OKT3 as the antigen, the IgG and IgM antibodies of all specificities to OKT3 were determined. Except for patient No. 18, the ELISA reactivity of the patient's pretreatment serum was similar to that of the 15 controls, both for IgG and for IgM (Table 1) and the anti-Ig with light chain reactivity gave essentially the same result as anti- γ chain.

Patient No. 18 had the highest pretreatment reactivity, which was exclusively IgM. This binding could be completely eliminated by preincubation of the test serum with BALB/c serum, and partially blocked with preincubation in normal goat serum. A rheumatoid factor assay with human IgG was within normal limits (<60 I.U.). The level of the IgM antimouse IgG antibody did not change by more than 5% during treatment, and the patient responded normally to the OKT3 therapy (Table 2).

An anti-OKT3 antibody response, defined as doubling of the pretreatment OD₄₀₅, was detectable at various times posttreatment in 14 (70%) of those individuals (Tables 2 and 3). Four had only IgM and one had only IgG. The IgG was detected significantly later than the IgM by an average of 10 days, although the time of the peak level was the same (20–23 days) (Tables 2–4). Antibodies were detected during OKT3 administration in 8 patients. All 8 had IgM antibodies and 2 also had IgG. Only one patient (No. 4), who had IgG as well as IgM anti-OKT3, showed resistance to the pharmacologic effects of OKT3. The other 7 cleared their T cells normally despite the presence of IgM anti-OKT3. Only 10% (IgG) and 33% (IgM) of the responders had persistent antibodies when checked 6 weeks or longer after the last dose. Two had detectable levels 14 weeks posttherapy (Nos. 7, 15).

Enhancing assay. This assay was used to detect isotype and light chain reactivity by assaying T8⁺ and T4⁺ PHA blasts coated with OKT8, OKT4, or OKT3 antibodies. Three patients (Nos. 10, 12, and 14) had pretreatment reactivity above the 95th percentile (Tables 1 and 2). Of the 16 tested, 7 (44%) showed a two-fold or greater increase in reactivity to OKT8 (IgG_{20k})-coated cells. Only two (12%) bound (both weakly) to OKT4 (IgG_{20k})-coated cells, suggesting that the reactivity detected was primarily to isotype specificities on the heavy chain. The timing of the anti-isotype was closer to that of the IgM than the IgG (Table 4).

OKT8 and OKT3 enhancing reactivity were disparate in 2 patients. The serum from patient No. 1 reacted with OKT8 coated, but not OKT3 coated cells. She had blocking anti-Id antibodies and it is possible that these displaced the OKT3 from the target cell, giving a false negative. A similar phenomenon may explain the negative OKT3-enhancing results in patients Nos. 2 and 17 who also had blocking anti-Id. A second patient (No. 14) had the opposite pattern, reacting with OKT3-coated, but not OKT8-coated cells. The restricted reactivity in this case may have been due to anti-Id of the nonblocking type.

Blocking anti-Id assay. None of the pretreatment sera specifically blocked the binding of F-OKT3 or F-OKT8 to normal lymphocytes (Table 1). However, after the course of OKT3 therapy, 12 patients (60%) developed antibodies that selectively blocked the antigen-binding of F-OKT3. The sera blocked F-OKT3 in the presence of normal BALB/c serum and did not block F-OKT8 or F-anti-Leu 4, arguing that the specificity was restricted to idiotypic—and not allotypic or isotypic—determinants on OKT3 (Fig. 2).

TABLE 2. Onset of anti-OKT3 antibodies and effect on OKT3 response^a

Patient	ELISA-OKT3		Block- ing Anti- Id	Enhancing			Response to OKT3 (cells/ mm ³) ^d	
	IgG	IgM		OKT3	OKT8	OKT4	T3	T4+T8
1	19	4	8	—	8	—	3	153
2	15	—4	25	—	—	—	15	276
3 ^b	—	—	—	—	—	—	7	402
4	0	—2	0	1	1	—	477	524
5	11	7	2	2	7	—	0	41
6	—	8	5	1	1	—	0	153
7	—	—	—	—	—	—	1	118
8	5	—3	2	2	2	—	3	191
9	6	—8	10	4	4	—	0	130
10	—	—	—	p [—]	p [—]	p [—]	5	56
11	1	28	—	—	—	45	5	80
12	—	—	—	p [—]	p [—]	—	0	34
13	—	2	2	2	2	72	1	392
14	31	—10	8	p ³	p [—]	p [—]	16	379
15	—1	—5	—	—	—	—	23	242
16	—	—	—	—	—	—	4	129
17	—	—	14	—	—	—	17	436
18	10	p [—]	8	—	—	—	17	274
19	—	—10	—	—	—	—	30	231
20	—	—6	45	—	—	—	24	598
21	—	—	—	—	—	—	1	105

^aThe day when antibody was first detected (0 = last day of OKT3 therapy; negative numbers—days before therapy ended).

^bOnly 3 posttreatment samples.

^cAbbreviations: p = pretreatment sample positive, >95th percentile of normal controls; — = negative; blank = not done.

^dLast blood values on OKT3.

TABLE 1. Pretreatment reactivity

	ELISA (OD ₄₀₅) ^a			Enhancing assay (% increase)			Blocking assay (% inhibition)	
	Ig	IgG	IgM	OKT3	OKT8	OKT4	OKT3	OKT8
Patients	369 \pm 219	339 \pm 147	60 \pm 92	59 \pm 76	144 \pm 297	38 \pm 80	7 \pm 7	8 \pm 11
Controls	322 \pm 125		198 \pm 151	29 \pm 30	27 \pm 34	16 \pm 21		

^aExcludes patient No. 18 whose values were 1384 (Ig), 118 (IgG), and 1718 (IgM) on the 3 ELISA assays. Ig was a screening test using goat antihuman immunoglobulin reactive to γ -heavy chain and both light chains, while IgG and IgM were detected by heavy chain-specific antisera.

TABLE 3. Maximum antibody level^a

Patient	ELISA-OKT3: OD ₄₀₅		Blocking:	Enhancing (% increase MFC)		
	IgG	IgM	Anti-Id (% Inhibition)	OKT3	OKT8	OKT4
1	634(19)	596(59) ^b	75(8)	-	1122(19)	-
2	1761(15)	142(75)	89(25)	-	-	-
3	-	-	-	-	-	-
4	1314(14)	460(5)	83(14)	358(1)	569(2)	-
5	1723(15)	226(10)	91(12)	489(7)	178(7)	-
6	-	292(8)	92(14)	1116(14)	1183(19)	-
7	-	-	-	-	-	-
8	1971(12)	5583(12)	94(8)	640(22)	200(22)	-
9	782(10)	881(10)	33(10)	400(13)	263(13)	-
10	-	-	-	-	-	-
11	599(39)	608(45)	-	-	-	94(52)
12	-	-	-	-	-	-
13	-	869(13)	89(2)	1200(76)	1500(76)	67(76)
14	900(55)	538(29)	55(14)	1067(3)	-	-
15	655(-1)	385(-1)	-	-	-	-
16	-	-	-	-	-	-
17	-	-	88(22)	-	-	-
18	865(30)	-	92(16)	-	-	-
19	-	597(0)	-	-	-	-
20	-	199(45)	58(45)	-	-	-
21	-	-	-	-	-	-

^a Day of maximum (after OKT3 therapy ended) in parentheses.

^b Assays in italics were positive in the last sample tested (more than 6 weeks from the last OKT3 dose).

TABLE 4. Summary of time course of antibody response

	Number	Onset ^a	Peak ^a
IgM	13	0±3 ^b	23±7
IgG	10	10±3	20±5
Idiotypic	12	11±4	16±3
Isotype	7	4±1	23±9

^a Day after last OKT3 dose, mean ± SE.

^b Precedes IgG, P<.02.

Further experiments were performed to document whether the effect was indeed due to anti-Id antibodies. The presence of residual free OKT3 in the serum was ruled out by the lack of detectable mouse Ig on normal lymphocytes after incubation in the blocking serum. In all patients OKT3 antibody was undetectable in the serum by 2 days after therapy had ended (data not shown). For this reason only sera taken at least 48 hr after the end of therapy were analyzed (except for patient No. 4 who was demonstrated to clear the OKT3 within 7 hr). Serum that blocked OKT3 did not block F-anti-Leu 4, an IgG₁ antibody to a different epitope on the same molecule (Fig. 2). This argues strongly against free or complexed T3 antigen itself as a significant source of antigenic competition in the blocking assay.

Sera from two of the patients were analyzed by affinity chromatography using columns with immobilized OKT3 or normal mouse IgG. The sera from patients Nos. 4 (Fig. 2 and 3) and 8 (Figs. 4 and 5) were absorbed with mouse IgG on Sepharose. The effluent and the dialyzed 3M KSCN eluate were tested for blocking activity. The eluate from normal mouse IgG (patient No. 8, day 5) showed equivalent blocking of F-

absence of BALB/c serum. The analogous eluate from patient No. 4 had no blocking activity. In contrast, the effluent blocked F-OKT3 completely but had little activity against F-OKT8, even in the absence of added BALB/c serum (Fig. 4). The effluent (patient No. 4) and the starting serum were then absorbed on an OKT3 column. This removed the blocking activity, which was undetectable even after reconcentration. Taken together, these data indicate that the blocking activity was due to antibodies that reacted selectively with OKT3 and not to other mouse immunoglobulins, satisfying the criteria for anti-Id specificity.

The time course of the anti-Id antibody production was variable, but generally paralleled that of the IgG (Table 4). The anti-Id antibodies first became detectable at an average of 11 days (range 0-45) after therapy had ended. The most rapid antibody response was manifested at 12 days after the start of therapy in patient No. 4 (Fig. 3). At this time he became refractory to the T lymphopenic effect of OKT3. The only clinical signs of an allergic reaction were an eosinophilia of 12% and a fever upon administration of the OKT3. No serum sickness lesions (glomerulonephritis, vasculitis, mouse Ig deposits by immunofluorescence) were seen in renal biopsy taken during the OKT3 treatment. The patient suffered no adverse systemic effects from this response, although the rejection process became reactivated and the allograft was eventually rejected. This was the only patient whose therapy was stopped because of anti-OKT3 antibodies. Three other patients had anti-Id antibody in the first sample analyzed, 2 days after therapy ended. Anti-Id antibody persisted in 33% of patients after 6 weeks; the longest documented was 6 months (No. 8).

Of the 12 patients that made a blocking anti-Id antibody, 3 (25%) had no IgG and 2 (17%) had no IgM reactivity detectable by ELISA. Similarly, 30% of those with blocking anti-Id had no isotype/light chain reactivity detectable by the enhancing assay. Patient No. 17 made exclusively an anti-Id response, detectable only in the blocking assay. Furthermore, the anti-Id assay detected antibody on one or more occasions in 7 other patients when the other assays were negative. This can be seen, for example, in the time course of the antibody response in patient No. 8 (Fig 4), in which the anti-Id response was persistently positive when the ELISA and enhancing assays were negative. Two patients (Nos. 11 and 15) made anti-OKT3 antibody detected on ELISA that did not block antigen binding or have reactivity to OKT8. These antibodies may include nonblocking anti-Id.

To estimate the portion of the antibody response that was anti-Id, serum from patient No. 8 at the peak of the antibody response (day 12, Fig. 4) was analyzed by ELISA using normal BALB/c serum as a competitive inhibitor for non-anti-Id antibodies. Normal mouse serum (100 μl) was able to block 69% of the IgG binding to OKT3, while 100% could be blocked by 10 μg OKT3, so that anti-Id constituted about 31% of the reactivity.

Correlations with clinical course. With the exception of the one patient (No. 4) who developed anti-Id antibodies that neutralized the in vivo effectiveness of OKT3, no adverse effects were associated with the antimouse Ig or anti-OKT3 idiotype response. The response to therapy and outcome of the graft were not correlated with the presence or absence of an antibody response. However, of the 6 patients who received cyclophosphamide (Nos. 10 and 17-21) only 17% made an IgG

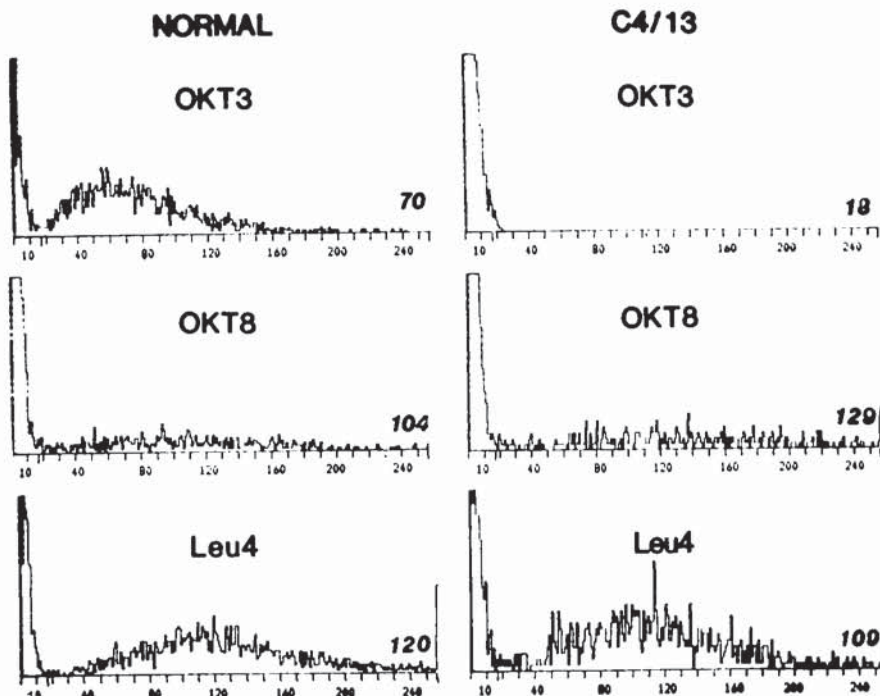


FIGURE 2. Fluorescence histograms of blocking anti-Id assay. Normal lymphocytes stained with fluoresceinated monoclonal antibodies in the presence of normal BALB/c serum and either normal human serum (left column) or serum from patient No. 4 (right column). The patient's serum blocks F-OKT3, but not F-OKT8 (both are IgG_{2a}) or F-anti-Leu 4 (IgG1, reactive to the T3 antigen). Cell number is on the y-axis and fluorescence intensity is on the x-axis. The number in the lower right of each histogram is the median fluorescence channel of the positive cells.

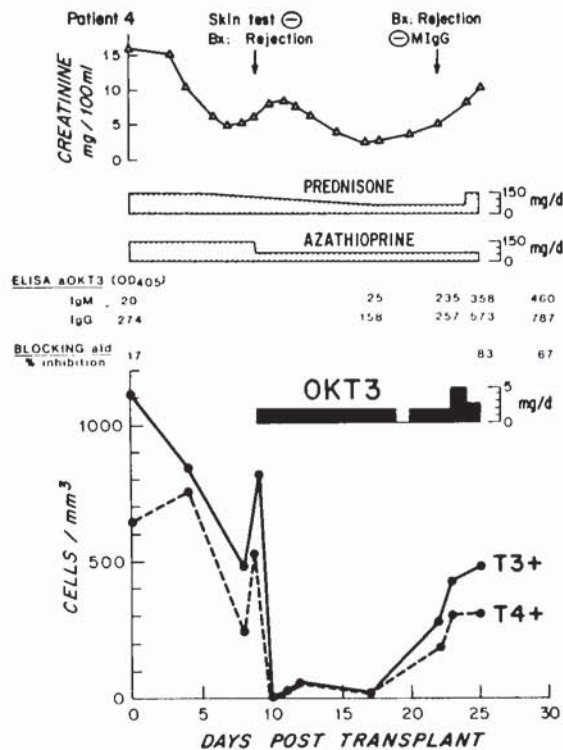


FIGURE 3. Clinical course of patient No. 4, who developed blocking anti-Id during therapy and became resistant to the T lymphopenic effects of OKT3. IgG and IgM anti-OKT3 was detected during therapy. Blocking anti-Id appearance was accompanied by resistance to the T lymphopenic response to OKT3 (day 22).

the non-cyclophosphamide group made such antibodies ($P < 0.076$). Cyclophosphamide had little effect on the blocking anti-Id response, which still occurred in 50% of patients—although somewhat later than the others, peaking at 16–45 days. No correlation with the recipient's HLA phenotype and

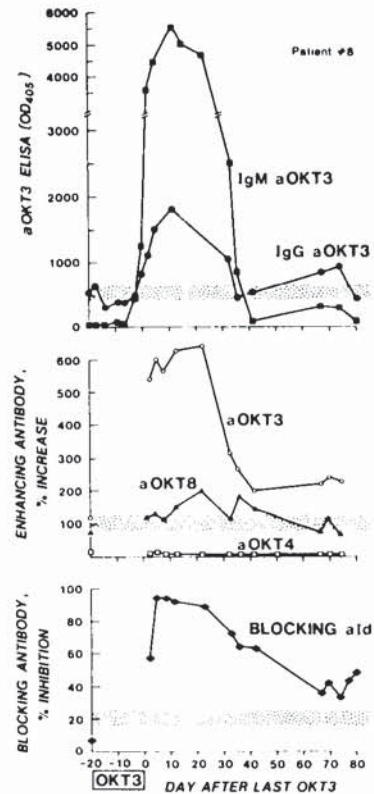


FIGURE 4. Time course of antibody response in patient No. 8, as detected by each assay in this study. The horizontal bar is the threshold of positivity (2 SD above the mean of normals). Note the early rise of IgM and the presence of both blocking anti-Id and anti-isotype (OKT8 enhancing assay). No antibodies to OKT4 were detectable.

DISCUSSION

In this series 60% of the patients treated with OKT3 developed anti-Id antibodies and 44% anti-isotypic antibodies, de-

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