Modulation of T-lymphocyte differentiation antigens: Potential relevance for multiple sclerosis

(monoclonal antibody/supporter cell/antigen modulation)

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ABSTRACT Effects of the anti-T-cell monoclonal antibodies OKT3, OKT5, and OKT8 on T-cell surface properties and cell functions were evaluated. Incubation of mononuclear cells isolated from peripheral blood for 48 hr with each monoclonal antibody in the absence of complement resulted in modulation of their respective surface antigens; i.e., the number of cells detected by immunofluorescence as positive for the T3, T5, and T8 surface antigens was reduced. T3, T5, and T8 antigens were not modulated independently. A radiolabeled second antibody technique confirmed modulation by OKT3 and OKT8 and indicated that T-cell differentiation antigens can regenerate in culture. Incubation of mononuclear cells with OKT3 increased the number of sheep erythrocyte-binding lymphocytes (E⁺-rosetting cells) and markedly increased the number of avidly E⁺-rosetting cells. Incubation with OKT8 reduced the number of E⁺- and of avidly E⁺-rosetting cells. OKT3 induced both mitogenic reactivity and suppressor cell activity; cells modulated by OKT8 exhibited reduced mitogenic reactivity and reduced suppressor cell function. The decreases in total T cells, in avid T cells, in suppressor cell number, and in suppressor cell function that follow modulation by OKT5 mimic changes observed in multiple sclerosis patients.

T-cell subset counts in blood and T-cell subset functions as detected by in vitro assays are aberrant in several diseases suspected to be autoimmune processes. During attacks of multiple sclerosis (MS), circulating T cells, whether defined as cells that form rosettes with sheep erythrocytes (E⁺-rosetting cells) or as cells that bear the T3 surface differentiation antigen, are decreased in number (1–6). During both stable and active phases of MS, the number of avid or early E⁺-rosetting cells is likewise reduced (7–9). The T suppressor/cytotoxic (T₈) cell subset, detected by presence on the cell surface of the differentiation antigens T5 and T8, is decreased during attacks of MS, as is the suppressor cell containing the T₈ cell subset recognized by the presence of Fe receptors for IgG (5, 6, 10). Finally, suppressor cell function measured in vitro is defective during active MS (11–13).

Mechanisms to explain this array of changes in T-cell number and function in MS include destruction of discrete T-cell subsets and modulation of T-cell surface antigens. In MS, antibodies that induce complement-dependent lysis in vitro are sometimes found but no correlation with disease activity has been shown (14–16). Whether such antibodies lyse T₈ cells selectively, as has been shown to be the case in systemic lupus erythematosus (17, 18), is not known. In vitro lysis, when mediated by disease-associated antibodies, is greater at room than at body temperature. As with disease-associated antibodies (17–19), monoclonal antibody (mAb) to T₈ cells (e.g., OKT8) can induce complement mediated lysis of T₈ cells in culture (20).

Antibodies may, under certain circumstances, modulate cell surface antigens without lysing the cells (21–23). This mechanism, which may be important in vivo, involves antibody interaction with cell surface antigen, redistribution of antigen-antibody complexes (capping), and endo- or exocytosis. The process requires a metabolically active cell. Modulation of a T-cell subset-confined differentiation antigen could remove the very marker that permits detection of the subset. Thus, modulation might result in apparent underrepresentation of the number of cells in a given subset.

Interaction of antibody with cell surface antigens may also affect cell function. The mAb OKT3, which is a marker for the total T-cell population, induces profound mitogenic effects (24) whereas the T₈-specific mAbs OKT5 and OKT8 inhibit concavalin A (Con A)- and phytohemagglutinin-induced mitogenic reactivity (25). mAbs directed against T₈ cells will also, in the absence of complement, inhibit cytolytic activity (25–27).

We have evaluated the capacity of the anti-T-cell mAbs OKT3, OKT5, and OKT8 to modulate their respective surface differentiation antigens and have studied the effects of modulation on both cell membrane properties and cell function.

METHODS

Blood Donors. Venous blood from healthy young adults (20–40 years old) was collected into B–D Vacutainer tubes containing acid/citrate/dextrose solution (Becton–Dickinson).

Monoclonal Antibodies. OKT3 and OKT8 mAbs were purchased from Ortho Laboratories. Most OKT8 samples contained sodium azide; some did not. OKT5 was provided by P. C. Kung of Ortho Laboratories.

Modulation of Cells with OKT mAb. Peripheral blood mononuclear cells (MNCs) were separated by the Ficoll/Hypaque method; 10⁷ pelleted cells were suspended in 200 μl of mAb solution (5 μg/ml for OKT8, 2.5 μg/ml for OKT3, and 10 μg/ml for OKT5) and incubated for 30 min at 0°C. After incubation, 10 ml of medium (RPMI/20% heat-inactivated fetal bovine serum containing gentamycin at 100 μg/100 ml) was added. Cells in medium were then cultured for 48 hr at 37°C in humidified 5% CO₂/95% air. Cells treated as described above will be referred to as modulated cells. “Culture control” cells were similarly kept at 0°C for 30 min but without added mAb, suspended in 10 ml of medium, and cultured for 48 hr.

Abbreviations: MS, multiple sclerosis; T₈, T suppressor lymphocytes; E⁺-rosetting cells, sheep erythrocyte-binding lymphocytes; T₅, T cells bearing receptors for the Fe portion of IgG; mAb, monoclonal antibody; MNC, mononuclear cells; Con A, concavalin A; HBSS, Hank’s balanced salt solution; FITC, fluorescein isothiocyanate; GAM-IgG, goat anti-mouse IgG.

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Immunofluorescence Assay. Freshly isolated, modulated, or control MNCs (10^6) were pelleted and suspended in 25 μl of mAb (2.5 μg/ml) solution for 30 min at 0°C. mAbs were diluted in Hanks' balanced salt solution (HBSS)/1% bovine serum albumin. Cells were washed three times in HBSS/1% bovine serum albumin and incubated for 30 min at 0°C with fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG. Cells were fixed and mounted on slides, and 200 cells were assayed for fluorescence, using a Leitz Ortholux microscope. All samples were coded prior to reading.

125I-Labeled Goat Anti-Mouse IgG (GAM-IgG) Binding Assay. One hundred micrograms of IgG isolated from a goat antihuman serum (Nordic, El Toro, CA) by ammonium sulfate precipitation and DEAE chromatography was iodinated by the lactoperoxidase method of Marchalonis (28). Free iodide was separated by Sephadex G-25 chromatography. The 125I-labeled GAM-IgG was diluted in HBSS/1% bovine serum albumin/10% goat serum containing a large excess of unlabeled iodide, so that a 25-μl aliquot contained 20,000 cpm. MNCs, either freshly isolated, modulated, or from control cultures, were pelleted and incubated with mAb at 2.5 μg/ml as in the immunofluorescence assay. The cells were washed three times and incubated with 25 μl of 125I-labeled GAM-IgG for 30 min at 0°C. The cells were again washed three times, the supernatant was aspirated, and the cell pellet was assayed in a gamma counter. Specific binding of 125I-labeled GAM-IgG to cell-bound OKT mAb was determined by subtracting cpm bound in the absence of mAb from cpm bound in its presence.

T Rosette Determination. Total E+ rosetting cells and avid E+ rosetting cells were enumerated as reported (7) except that macrophages were not removed from the MNC preparation.

Assays of Lymphocyte Function. Mitogenesis and suppressor cell function induced by mAb or Con A. Our methods to determine mitogen response and suppressor cell function have been reported (11). Samples of pelleted MNCs were incubated for 30 min with mAb as described for the modulation procedure, suspended at 10^6 cells per ml, and cultured in flasks (Falcon) for 96 hr. Other MNC samples were cultured with Con A at 3 μg/ml or in medium alone for 96 hr. Mitogenesis was determined by pulsing aliquots with [3H]thymidine for 5 hr.

Suppressor cell activity was assayed on the mAb-treated, Con A-stimulated, and control cultures described above. After 96 hr, cells from each culture were treated with mitomycin C (25 μg/ml) and washed, and 10^5 cells in 100-μl aliquots from each sample were recultured with 10^5 fresh autologous or heterologous responder cells also in 100 μl of medium. The new culture medium contained Con A at a final concentration of 3 μg/ml. Seventy-two hours later, [3H]thymidine uptake from quadruplicate cultures was measured after a 5-hr pulse.

Percent suppression induced by mAb or Con A was calculated as

\[
1 - \frac{\text{cpm of (Con A-induced responder cells + Con A-stimulated or mAb-treated cells)}}{\text{cpm of (Con A-induced responder cells + control cells)}} \times 100.
\]

Mitogenic capacity and suppressor cell activity of mAb-modulated cells. To determine the Con A-induced mitogenic capacity of mAb-modulated or control culture cells, MNCs were suspended after their initial 48 hr of culture in fresh medium containing Con A (3 μg/ml) and cultured for a further 48 hr. Cell aliquots taken from each culture flask were then pulsed for 5 hr with [3H]thymidine.

![Chart](chart.png)

**Fig. 1.** Effect of modulation of MNCs with OKT3, OKT5, or OKT8 mAb on surface properties of T cells. Results are mean ± SEM for the number of individuals tested. (A) Total E+ rosettes. (B) Avid rosettes. (C) T3+ cells. (D) T8+ cells. (E) T3+ T8+ cells. (F) Freshly isolated MNCs. (G) control cultures (48 hr, no mAb); (H) OKT3 modulated (48 hr); (I) OKT5 modulated (48 hr); (J) OKT8 modulated (48 hr).
Con A-induced suppressor cell activity of mAb-modulated and control culture cells was determined by culturing modulated and control cells for an additional 48 hr in fresh medium with or without Con A at 3 μg/ml. After 48 hr, cells from each culture were treated with mitomycin C and cultured for a further 72 hr with fresh responder cells and Con A as described for the Con A-induced suppressor cell assay.

Nonactivated mitomycin-treated modulated and control culture cells did not inhibit [3H]thymidine uptake by responder cells cultured with Con A. Accordingly, percent suppression induced by Con A-activated modulated cells could be calculated as

\[
1 - \frac{\text{cpm of (Con A-induced responder cells + Con A-activated modulated cells)}}{\text{cpm of (Con A-induced responder cells + nonactivated modulated cells)}} \times 100
\]

and compared directly to percent suppression induced by Con A-stimulated control culture cells calculated as

\[
1 - \frac{\text{cpm of (Con A-induced responder cells + Con A-activated control culture cells)}}{\text{cpm of (Con A-induced responder cells + nonactivated control culture cells)}} \times 100.
\]

Data were analyzed by using both Student's t test and the paired t test.

**RESULTS**

**Effects of Anti-T-Cell mAbs on Cell Surface Properties.**

**Incubation with OKT3 mAb.** Cell recovery from OKT3-modulated and control cultures did not differ significantly; the mean number of OKT3-modulated cells recovered was 112 ± 18% of control values \((n = 3)\). OKT3 modulation increased the proportion of E+ -rosetting cells to 65 ± 3% vs. 53 ± 4% for control cultures \((P < 0.05; \text{Fig. 1A})\) and more than tripled the proportion of avidly E+ -rosetting cells \((45 ± 4% \text{ vs. } 12 ± 2%; P < 0.001; \text{Fig. 1B})\). As shown in Fig. 1C, control cultures exhibited an increased percent of T3-positive cells \((76 ± 2)\) when compared with freshly isolated MNCs \((61 ± 5; P < 0.05)\) whereas, for OKT3-modulated MNCs, the percent T3-positive cells detected was decreased \((39 ± 6; P < 0.001)\). OKT3 induced marked blastogenesis (see below); the proportion of T3-positive cells in blast and nonblast populations was similar. Culturing MNCs with OKT3 did not alter the percent of T5- or T8-positive cells (Fig. 1D and E). T5- and T8-negative cells were found in both blast and nonblast populations.

**Incubation with OKT5 or OKT8 mAb.** Cell recovery from cultures containing OKT5 and OKT8 and control cultures did not differ \(100 ± 8\% (n = 4)\) for OKT5 and 93 ± 8% \((n = 5)\) for OKT8 vs. control cultures). OKT8 modulation reduced the proportion of E+ -rosetting cells to 43 ± 4% vs. 51 ± 3% for paired control cultures \((P < 0.05, \text{paired t test; Fig. 1A})\), reduced avidly E+ -rosetting cells to 8 ± 2% vs. 12 ± 2% \((P < 0.01; \text{Fig. 1B})\), and reduced T3-positive cells to 70 ± 2% vs. 76 ± 2% \((P < 0.02; \text{Fig. 1C})\). Similar trends were seen after incubation with OKT5; the limited amount of OKT5 available precluded detailed assessment.

The percent of MNCs positive for T5 and for T8 did not change significantly in control cultures (Fig. 1D and E). After OKT5 modulation, the percent of T5-positive cells detected was reduced to 7 ± 2 vs. 21 ± 3 for control cultures \((P < 0.001)\). OKT5 modulation did not change the percent of T8-positive cells. Less than 3% of OKT5-modulated MNCs fluoresced when stained directly with FITC-labeled GAM-IgG.

With OKT8 incubation, the percent of T8 positive cells was reduced to 17 ± 2 compared with 27 ± 2 for controls \((P < 0.001, \text{paired t test})\). Results from individual donors are given in Table 1. Analysis of results obtained by using OKT8 with and without Na3V indicate that the azide does not significantly affect the results. T8-positive cells from OKT8-modulated cultures appeared dimmer than the T3-positive cells from control cultures, suggesting that even positive cells had been partially modulated. When OKT8-modulated cells were stained directly with FITC-labeled GAM-IgG, 11 ± 3% were found to be positive—i.e., substantial mAb remained membrane bound.

**Quantitation of Modulation by Using 125I-Labeled GAM-IgG.** As seen in Table 2, OKT3-specific 125I-labeled GAM-IgG binding to OKT3-modulated cells was less than that to control cultures, in keeping with the results obtained in immunofluorescence studies. When the same experiment was done using OKT8, reduced OKT8-specific binding to OKT8-modulated cells was found.

As shown in Table 3, when modulated cells were washed

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<th>Table 1. Modulation of T8 antigen by OKT8 mAb and effect on Con A-induced suppressor cell activity</th>
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<td>% T8-positive cells</td>
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26 ± 2 | 16 ± 2 | 27 ± 5 | 20 ± 5 | 43 ± 6 | 29 ± 5 |

\(P < 0.001\) \(P < 0.05\) \(P < 0.02\)

T8-positive cells were identified by immunofluorescence. Each pair of values (control and modulated) indicates results from cells of a single donor. Donors are identified by letters in parentheses. \(F\) values were calculated by paired t tests.

* Modulation with OKT8/azide not studied by immunofluorescence.
repeatedly and then cultured for an additional 48 hr in antibody-free medium. OKT3 and OKT8 specific binding of ¹²⁵I-labeled GAM-IgG increased 2- to 20-fold, indicating that modulated antigens had been regenerated.

Effects of Anti-T-Cell Subset mAbs on T-Cell Function. Incubation with OKT3. OKT3 induced mitogenesis. After 96 hr in culture with OKT3, [³H]thymidine incorporation was 98,836 ± 33,142 cpm (mean ± SEM for three individuals) vs. 70,404 ± 24,595 (mean ± SEM) for cells from the same individuals incubated with Con A. OKT3 also induced suppressor cell activity as intense as that induced with Con A (41 ± 11% (n = 4) with OKT3 compared with 41 ± 7% (n = 7) with Con A).

Incubation with OKT5 or OKT7. OKT5 and OKT7 did not induce cell proliferation after 96 hr as measured by [³H]thymidine incorporation [1,360 ± 477 cpm (n = 6) for OKT5 and 1,033 ± 498 cpm (n = 9) for OKT7 compared with 1,267 ± 716 (n = 10) for controls] and OKT7 did not induce suppressor cell activity [mean suppression −1 ± 8% (n = 6)]. When OKT8-modulated MNCs were further cultured in fresh medium containing Con A, [³H]thymidine uptake at 48 hr [36,912 ± 7,482 cpm (n = 11)] was less than that of control culture MNCs (44,138 ± 7,482 cpm; P < 0.05, paired t test). Reduced [³H]thymidine uptake was found in 12 of 16 donors. When OKT8-modulated MNCs were cultured with Con A and the suppressor cell activity thus induced was assayed, the suppression observed was reduced significantly (23 ± 5%) from that seen with control culture cells handled in the same way (43 ± 6%; P < 0.02, paired t test; see Table 1). In a limited number of studies, no reduction in suppressor function was found to follow OKT8 modulation (Table 4).

DISCUSSION

By using immunofluorescence techniques, we have found that modulation of T3, T5, and, to a lesser extent, T8 antigens occurs when cells are cultured with these mAbs. Modulation of antigen by specific antibody has been observed in a wide variety of antigen–antibody systems (21–23). In addition, exogenous agents such as viruses can modulate cell surface antigens (29).

T3 antigen is present on (virtually) all T cells and labeling of cells with OKT3 has become one of the standard procedures for recognizing T cells. Modulation of T cells with OKT3 increased the total number of E−-rosetting cells but reduced the number of T3-positive cells, thus confirming that T3 is distinct from the E−-rosette receptor. Recently, a mAb recognizing the E− receptor has been described (30). The discrepancy between T-cell numbers obtained by using mAb and E−-rosetting techniques points up the desirability of using more than one marker system when enumerating cell types. Incubation with OKT3 also markedly increased the number of avidly E−-rosetting cells (31), a result exactly opposite to that found in M5 patients (7–9).

Enumeration studies after modulation with OKT5 and OKT8 mAbs define differences between these two antigens, both of which are considered to be markers for T5 cells. Modulation of the T5 differentiation antigen did not alter the number of T8-positive cells as measured by immunofluorescence and vice versa. The independent modulation of T5 and T8 indicates that an antibody may interact selectively with only one of these antigens on a single cell. Possibly, in man, some cells express only one of these antigens; malignant cell lines expressing T8 but not T5 have been described (20). Studies of normal individuals do show differences with respect to numbers of circulating T5- and T8-positive MNCs (unpublished observations; D. W. Paty, personal communication). In studies of M5 patients with active disease, depressed numbers of T5 cells are reported when either OKT5 or OKT8 are used to enumerate the cells (5, 6) but not when anti-Leu 2A antibody, another T5 cell marker, is used (32).

The numbers of total T cells as defined by E− rosettes or by labeling with OKT3 and of avidly E−-rosetting cells were reduced by modulation with OKT8. The overall numbers of T cells as defined by the E−-rosette technique or by the T3 antigen are modestly reduced in the circulation in M5 patients. The reduction in avidly E−-rosetting T cells in M5 patients is more profound. Thus, modulation of the T8 antigen results in three distinct changes characteristic of M5.

Our functional studies confirm the marked mitogenic properties of mAb OKT3 (24). Data obtained by serial flow cytometric analysis indicate that OKT3 evokes an earlier response and induces a higher proportion of cells into the cell cycle than does Con A (unpublished data). In addition, we show that OKT3 induces suppressor cell activity. Whether the mechanism of this activation is the same as that for mitogen-induced suppressor activity remains to be determined.

OKT8-modulated cells exhibited both reduced mitogenic response to Con A and reduced Con A-induced suppressor cell...
activity. Our finding of reduced mitogenic reactivity after OKT8 modulation agrees with the results of Platoucas and Good (25). These workers found that direct addition of OKT8 to MNCs reduced response to Con A. The effect of OKT8 on functional suppressor activity shown here should be compared with their finding (25) that addition of OKT8 to cytolytic cells generated in mixed lymphocyte reactions blocked cytolytic activity. Cytolytic and suppressor activity may be mediated by identical T-cell subsets (25-27).

As an additional measure of modulation, a radiolabeled second antibody technique was used. This semiquantitative technique confirmed that OKT3 modulates T3 and that OKT8 modulates T8. By using the radiolabeled technique, we showed that the amount of T3 and T8 antigens present on cells rebounds in culture once the mAb has been removed from the medium. This finding establishes that, as reported in other systems (21, 22), modulation is reversible.

Our observations on the effects of mAbs directed against T-cell differentiation antigens invite comparison with T-cell alterations observed in MS patients. In MS, response to T-cell mitogens is modestly reduced (33). Suppressor cell function and suppressor cell number, as defined by OKT5 and OKT8 antibodies, are reduced when disease is active. Our data show that a mAb directed against T8 antigen reduces mitogenic reactivity, albeit modestly, and suppressor cell function as well. Antibodies directed against T5 and T8 can also lead to underestimation of suppressor cell number subset. When MNCs from MS patients are kept in culture for 48 hr, the number of T8-positive cells increases, a finding that supports the postulate that in vivo modulation may be occurring (unpublished results). Autoantibodies to lymphocytes, as determined both by immunofluorescence and complement-dependent cytolysis, have been shown to be increased in some MS patients (14-16). Their role in the disease remains to be defined; possibly, they modulate surface antigens, including T8. We have presented evidence that mAb OKT8 crossreacts with oligodendroglial cells, the putative cell target in MS, and suggested that antibodies directed against antigens shared by oligodendroglial cells and by lymphoid cells may alter both cell types (34).

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