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COMPLEMENT RECEPTORS (CR) AND CYTOTOXIC RESPONSES: MONOCLONAL ANTIBODIES DIRECTED AGAINST CR1 AND CR3 INHIBIT THE GENERATION OF HUMAN ALLOSPECIFIC AND VIRUS SPECIFIC CYTOTOXIC CELLS IN VITRO.

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ABSTRACT

A variety of cellular immune responses involve complement factors which bind to specific receptors, and modulate or effect a specific reaction. Monoclonal antibodies (MAb) have been generated against complement receptors (CR) 1 and 3, which were utilized to investigate human allogeneic and Epstein-Barr virus specific cytotoxic cells in vitro. MAb OKM1, which binds to the C3bi CR (CR3), and MAb M710, which binds to the C3b/4b CR (CR1), inhibited the generation of both allogeneic and virus specific cytotoxic responses in vitro in a dose-dependent way; doses of 1 µg/ml (or greater) completely abrogated the cytotoxic responses. Inibition of these responses was observed when the MAb was added to the cultures at any time point except the last

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two days. In addition, treatment of the responder (but not the stimulator cells) with either MAb resulted in complete inhibition of cytotoxic responses. These experiment indicate that complement receptors participate in the generation of human cytotoxic responses in vitro.

INTRODUCTION

Complement factors have now been identified which actively participate in the regulation of various phases of the mammalian immune response (1). Specifically, C3, in relatively high doses, has been found to suppress human allogeneic cell-mediated lympholysis (CML) responses in vitro (2), while in smaller doses, C3, as well as its breakdown products C3b and C3d, enhances CML (3). C3, or its breakdown products, can modify early phases of the generation of cytotoxic T lymphocytes (CTL), but they have no effect on established CTL. C3 and its cleavage products (C3b, C3c, C3d) also have inhibitory effect on lymphocyte proliferation in response to antigens and mitogens (3,4,5).

MNC carry surface membrane receptors for different complement factors including C3b (CR1), C3bi (CR3) and C3d (CR2). CR1 is also present on the surface of erythrocytes, macrophages and lymphocytes, while CR2 is expressed by B lymphocytes, and CR3 can be detected on erythrocytes, macrophages, neutrophils, lymphocytes and natural killer cells (6).

Human allogeneic MNC co-cultured in vitro generate specific cytotoxic cells able to kill target cells bearing HLA antigens related to those of the stimulator individual (7). In addition, stimulation of human mononuclear cells (MNC) with autologous Epstein-Barr virus (EBV) infected B cell lines leads to the generation of virus and HLA

restricted cytotoxic cells within 10 to 14 days (8,9). The recent availability of MAb directed against various complement receptors (in particular CR1 and CR3) allowed evaluation of CR inhibition (via MAb binding), and the subsequent effects on the generation of human allogeneic and EBV-specific cytotoxic responses.

Indeed, MAb directed against complement factors CR1 and CR3 were used to evaluate the participation of each factor in the generation of cytotoxic responses in vitro. The addition of these MAbs during various phases of cellular response allowed evaluation of the temporal role of each MAb in the cytotoxic response.

MATERIALS AND METHODS

Mononuclear cell (MNC) isolation. MNC were obtained from heparinized human peripheral blood by standard Ficoll-Paque gradient centrifugation.

Cell-mediated lympholysis. Effector cells were obtained by harvesting MNC produced by a 6 day mixed lymphocyte culture (MLC). Responder cells (1 x 10^6/ml) and irradiated (2000 rads) stimulator cells (0.5 x 10^6/ml) were co-cultured in RPMI-1640 medium (containing 10% heat-inactivated normal human serum) in flat bottom 24-well plates (Costar, Cambridge, MA). Phytohemagglutinin-stimulated (1 µg/ml) MNC of the stimulator type (Burroughs-Wellcome, Beckenham, England) were used as target cells. Prior to use, these cells were labeled with ^{51}Cr by incubation with 200 µCi of ^{51}Cr-labeled sodium chromate (New England Nuclear Corp., Boston, MA). Effector and target cells were then incubated at 37°C (4 hr), before the supernatants were collected, and counted in a gamma counter. The percentage of specific cytotoxic chromium release was evaluated as follows: % cytotoxicity =

(experimental cpm - spontaneous cpm)/(maximum cpm - spontaneous cpm) x 100. Spontaneous release in these experiments ranged between 18 and 25% within 4 hours. MNC cultured without stimulator cells served as unsensitized control effector cells; the specific lysis obtained from unsensitized cells ranged from -1 to 6% , which was subtracted from the percentage of lysis obtained by sensitized cells.

Generation of EBV-lines. Briefly, the supernatant containing EBV was obtained by culturing the B-95 cell line in RPMI-1640 with 10% fetal calf serum and antibiotics (see below) for 10 days at 37°C in the presence of a humidified atmosphere with 5% CO_2. The cells were harvested and centrifuged (30' X 400g) and the supernatant was collected, filtered with 45 millipore filter (Gelman, Ann Arbor, MI) and aliquoted in 1 ml tubes. Ten million MNC (1 x 10^6/ml) in RPMI-1640 supplemented with serum as described above, were infected with Epstein-Barr virus (EBV) (2 ml of supernatant for 50 X 10^6 MNC) in the presence of 1 µg/ml of cyclosporin A and left for 20 days at 37°C in a humidified atmosphere containing 5% CO_2. On the twentieth day the cells were washed three times and resuspended in 100 ml of media. Subsequently, every three days 1x 10^7 cells from each line were collected and refed with 100 ml of fresh medium.

Preparation of autologous effector cells. 2.5 x 10^6/ml MNC were cultured with 0.5 x 10^5 autologous radiated EBV-lymphocytes (2000 R Cesium source) for 10 days at 37°C in humidified atmosphere at 5% CO_2 in 24-well tissue culture plates (Costar). On the 10th day of culture the cells were harvested, washed three times in PBS (NIH Media Unit) and resuspended in RPMI-1640 plus 20% FCS at a final concentration of 10 x 10^6 cells/ml. Cell-mediated lympholysis assays were performed as

described above, except that autologous EBV-transformed cells were labeled with ^{51}Cr as target cells.

Monoclonal antibodies and purified mouse myeloma proteins. Several MAbs were used in this study including: OKM1 (IgG2b) obtained from Ortho Diagnostic Systems (Raritan, NJ, USA), M710 (IgG1) purchased from Dakopatts (Denmark). MAb Leu-M3, which recognizes a monocyte related antigen, and biotin-conjugated anti HLA-DR MAb were obtained from Becton and Dickinson (Mountain View, CA). Two purified mouse myeloma proteins, UPC-10 (IgG2a) and MOPC-104E (IgMλ) which were used as negative controls, were purchased from Litton Bionetics (Charleston,SC).

RESULTS

Effects of human allogeneic CML responses by anti-CR MAb. Two different MAb were used: 1) OKM1, which recognizes the receptor for C3bi (CR3) (11); and 2) M710, which recognizes the receptor for C3b (CR1) (12). Both MAb were able to completely inhibit the generation of human allogeneic CTL if added at concentrations of 1 μg/ml or greater, at the initiation of the cultures (Fig. 1). In Fig. 2, effector cells (originated from mixed lymphocyte cultures in the absence or in the presence of 1 μg/ml of the 2 MAb) were assayed at serial 2-fold dilutions against target cells. A significant inibition of the cytotoxic response was found when the cells were co-cultuted with each MAb. On the other hand, control myeloma proteins, UPC-10 and MOPC-104E, had no effect on the generation of cytotoxic cells.

Kinetics of the inhibition of CTL by anti-CR antibodies. In further experiments, 1 μg/ml of either MAb was added at the initiation of the mixed lymphocyte cultures, and at daily intervals thereafter. As

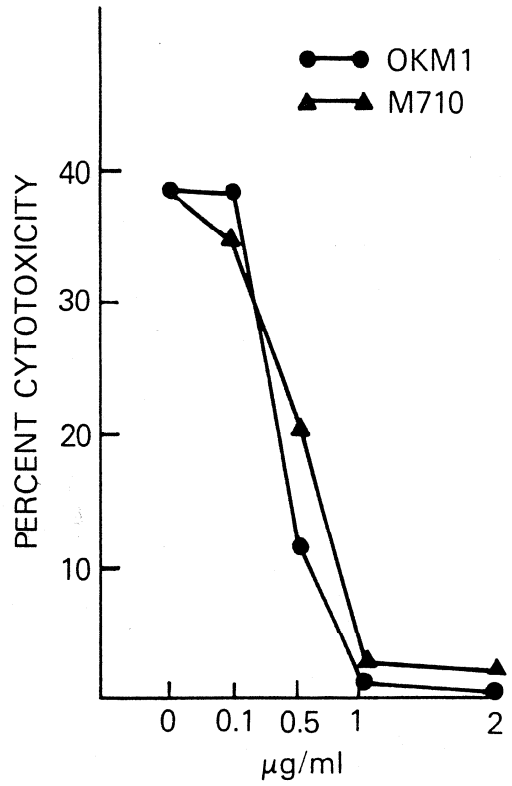

Fig. 1:

Inhibition of human allogeneic CML response by mouse MAbs directed against CR1 (M710), and CR3 (OKM1). MAbs were added at the indicated concentrations at the initiation of the cultures. Points represent the mean of 3 experiments at the effector:target ratio 100:1.

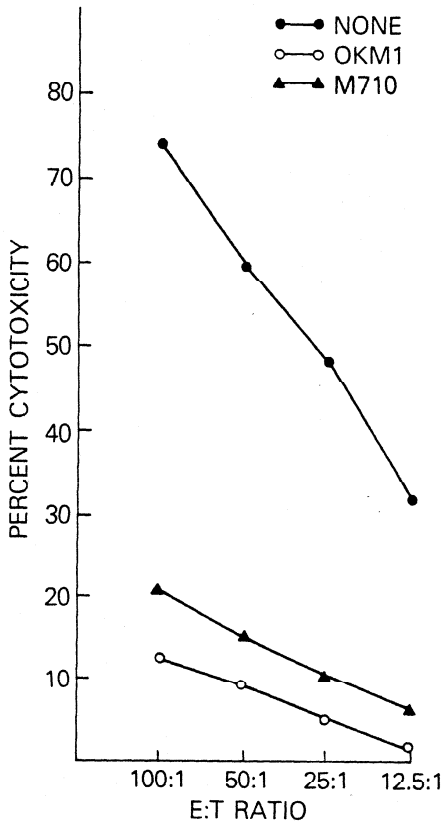

Fig. 2:

Inhibition of human allogeneic CML response by mouse MAbs directed against complement receptors. One µg/ml of each MAb was added at the initiation of the cultures.

shown in Fig. 3, CML responses were inhibited only if either MAb was added to the cultures during the first 3 days. Addition of either MAb on subsequent days failed to inhibit the CML responses, as did of 1 μg/ml of MAb added during the final 4 hours of the assay .

MAb effects on human EBV-specific cytotoxic cells. In vitro, incubation of human MNC with irradiated autologous B cell lines infected with EBV leads to the generation of HLA and virus restricted cytotoxic cells under the specified conditions if no MAb is added. The ability of the anti-CR MAb to block or otherwise modify the generation of these cytotoxic cells was evaluated (see Fig. 4). One μg/ml of each MAb completely inhibited the generation of cytotoxic cells; whereas MAb added at a concentration of 1 μg/ml during the final 4 hours chromium release assay had no effect. MAb directed against these CR inhibited the generation of virus specific cytotoxic cells, but failed to inhibit the function of differentiated cells or the conjugation of effector to target cells. Control myeloma proteins UPC-10 and MOPC-104E had no effect on the generation of EBV-specific cytotoxic cells (identical conditions).

MAb binding to responder and stimulator cells. In order to investigate the mechanism by which anti-CR MAb inhibit the generation of CTL we preincubated responder or stimulator cells with MAb prior to starting the mixed lymphocyte culture. As shown in Table I, pretreatment of the responder, but not of the stimulator cells, abrogates the generation of CTL. The anti-CR antibodies interfered with the initial presentation of the antigen to the responder cells, however, demonstrated no effect with the stimulater cells. DR antigens were necessary for the generation of CTL. Anti-DR antibodies blocked the

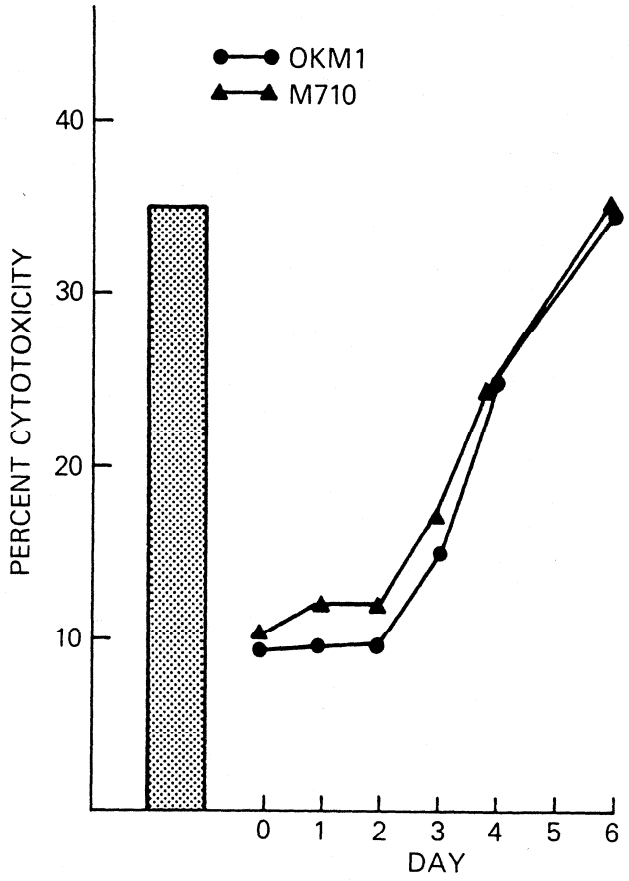

Fig. 3:

Mouse MAb directed against complement receptors inhibit human allogeneic CML if present during the generation phase of CTLs. One μg/ml of MAb was added at all time points. Vertical bar represents the control allogeneic CML response. Cultures were harvested and assayed on day six.

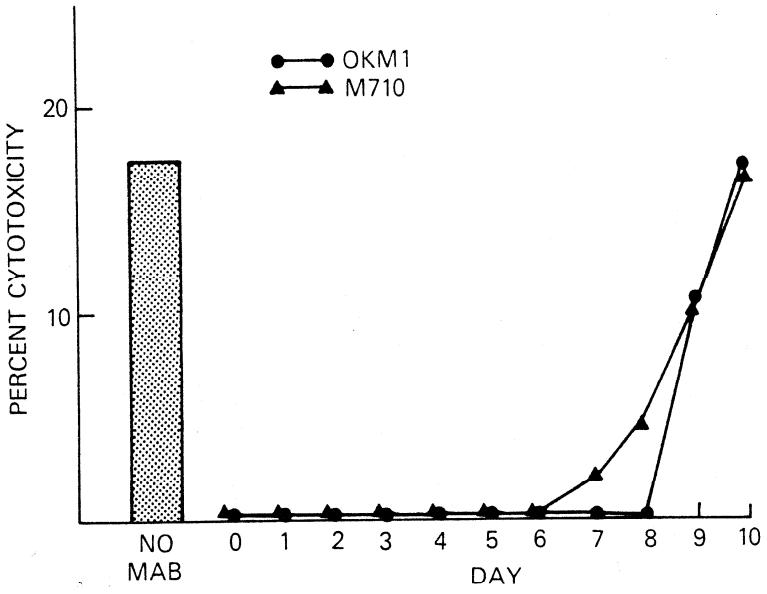

Fig. 4:

Inhibition of the generation of EBV specific cytotoxic cells in vitro by MAbs directed against complement receptors. One µg/ml of MAb were added at all indicated time points. Both MAb inhibit the generation of virus specific MAb if added up to 2 days prior to the final assay. MAb added during the final assay failed to inhibit cytotoxicity. Results from 2 experiments are shown at the effector:target ratio 50:1.

generation of CTL, similar to that observed with the anti-CR MAb. In contrast, an antibody directed against another monocyte related antigen, Leu M3, failed to inhibit the generation of CTL (Table II)

TABLE I

Pretreatment of the responder but not stimulator cell with OKM1 or M710 monoclonal antibodies abrogated the generation of cytotoxic cells.

Pretreated[a] Responder	Stimulator	Percent Lysis E:T 100:1
0	0	22.0
OKM1	0	6.1
0	OKM1	23.3
M710	0	8.2
0	M710	30.9

[a] Responder or stimulator cells were pretreated at 4°C for 1 hr with 2 µg/ml of the indicated monoclonal antibody.

TABLE II

OKM1 and anti-DR antibodies but not anti Leu M3 inhibit human allogeneic CML

Antibody[a]	Percent[b] Suppression
OKM1	95%
anti-DR	50%
anti-Leu M3	12%
UPC 10	9%
MOPC-104	8%

[a] 2 µg/ml of antibody were added at the initiations of the cultures.

[b] Percent reduction of the normal human allogeneic CML.

DISCUSSION

The biochemical structure of the CR have been recently investigated. It was found that the C3bi receptor, which is identified by the OKM1 MAb, consists of two covalently linked subunits which are structurally and antigenically related to the leucocyte function antigen (LFA)-1 (13). LFA-1 is an antigen which is necessary for the conjugation of effector and target cells. Anti-LFA-1 MAb inhibits the binding of specific murine effector cells to allogeneic and xenogeneic target cells, while anti-Mac-1 (OKM1) does not (13). The results of this study indicate that MAb OKM1 blocks early phases of CTL, but not the conjugation of effector and target cells. Analogies between the C3bi receptor and the LFA-1 antigen strongly indicate that CR are important in the functional expression of immune cells and their cytotoxic responses. The anti-CR MAbs OKM1 and M710 blocked the generation of CTL, but were unable to modify the late phases of CTL or inhibit the effector-target cell conjugation. The CR studied in these assays demonstrated a functional association with the antigen recognition structures on macrophages or the molecules necessary for the presentation of the processed antigen to the responding T lymphocytes.

The C3b receptor is a single-chain glycoprotein of estimated molecular weight 195-250 kd (14). C3b, bound to CR1 is known to modify both T and B cell function (3,15). This study supports the hypothesis that CR1 participates in the generation of CTL. CR1, which is present on macrophages as well as B lymphocytes, may be necessary for the recognition and presentation of antigens by these cells to the T lymphocytes. Alternatively, it is possible that anti-CR1 MAb might interfere with the function of a small subpopulation of the T cells

which have been recognized to express membrane receptors for the C3b (15).

In conclusion, MAb directed against the CR1 and CR3 inhibit the generation of allogeneic and virus specific CTL. Possible mechanisms of this inhibition are: 1) inhibition CTL via an inhibition of proliferative response, (both MAb inhibited the proliferative responses to alloantigens (data not presented)); 2) interference with the function of T lymphocytes; this is particularly true in the case of M710 since certain T lymphocytes carry CR1. The identification of patients with an OKM-1 deficiency who present with overwhelming infections and severe defects in raising cytotoxic responses provide a solid basis for the pathophysiologic significance of data presented herein (16,17).

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