

A MONOCLONAL ANTI-DC1 ANTIBODY SELECTIVELY
INHIBITS THE GENERATION OF EFFECTOR T CELLS
MEDIATING SPECIFIC CYTOLYTIC ACTIVITY*

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The human Ia region of the major histocompatibility complex (MHC) is composed of a cluster of genes in strong linkage disequilibrium with each other, coding for molecules similar in structure expressed on the surface of B lymphocytes, macrophages, and T cells, especially upon activation. Since the first locus, DR, was recognized in the VII Histocompatibility Workshop, direct evidence has been obtained for at least two more loci, DC and BR, possessing genetic markers defined by specific alloantisera (1). The products of the DR locus are the predominant species of Ia molecules, accounting for at least 60% of the Ia pool (2). There is no doubt that DR molecules play a role in the mixed lymphocyte reaction (MLR) (3). Recent studies have shown that proliferation of T cells can be strongly inhibited by pretreatment of either the responder T cells or the stimulating cells with monoclonal antibodies specific for products of the DR locus (4, 5).

The products of the DC locus constitute a smaller fraction of Ia molecules (2). They have been shown both by microfingerprinting (6) and by two-dimensional gel analysis (7) to differ from DR molecules in both subunits. These substantial structural differences suggested the possibility that DC molecules could have functional properties distinct from those of DR molecules. The availability of a monoclonal antibody (MAb) specifically directed against an allelic DC determinant, DC1 (nomenclature equivalent MB1, MT1, LB12), a DC determinant in strong linkage disequilibrium with DR1,2 and w6 (8), makes it possible to test this hypothesis.

In this study we have investigated the effect of the anti DC1 MAb on MLR and other in vitro systems. We show here that, unlike anti-DR antibodies, anti-DC1 has no effect on cell proliferation. However, anti-DC1 affects the generation of effector T cells mediating specific cytolytic activity, whereas no inhibitory effect can be observed on natural killer (NK) and antibody-dependent cytotoxic cell (ADCC) activities.

Materials and Methods

Monoclonal Antibodies. Derivation of the MAb used in the present study has been previously described (6, 9, 10). The anti-DR PTF29.12 is an IgG2 antibody to a nonpolymorphic determinant of human DR molecules; BT3/4 is an IgG1 antibody directed at a polymorphic determinant of DC molecules present on the DC1 allele (6). The MAb were used in the form of purified IgG at a final concentration of 50 µg/ml unless otherwise specified.

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Cell Cultures. Peripheral blood mononuclear cells (PBMC) were obtained from donors typed for DR and DC1 as previously described (11). The responding cell population in mixed lymphocyte culture (MLC) consisted either of PBMC or purified T lymphocytes obtained by isolating lymphocytes forming rosettes with neuraminidase-treated sheep erythrocytes (E-rosetting cells) by two sequential centrifugations on Ficoll-Hypaque density gradients. T cells were freed of erythrocytes by treatment with ammonium chloride buffer. The stimulating cell population, consisting of either PBMC or E rosette-depleted cells, was irradiated with 4,500 rad using a Cesium source. Unidirectional MLC were set up by culturing responding cells either in macrowells (10^6 cells/well) or in round-bottomed microwells (10^5 cells/well) with equal numbers of irradiated allogeneic cells in RPMI 1640 containing 10% fetal calf serum (FCS) as previously described (11). Control samples included responding or stimulating cells cultured alone. MAb were added at the onset of the culture, unless otherwise specified, at a dose of 5 μg /well, in the case of microcultures, or at a dose of 50 μg /well, when MLC were set up in macrowells. Cell proliferation and cytotoxicity were assessed on day 7 as previously described (12). Briefly, to evaluate cell proliferation, tritiated thymidine (^3H]TdR) was added to triplicate microwell cultures 18 h before harvesting. Cytolytic assays were performed against (a) phytohemagglutinin (PHA)-induced blasts derived from the stimulator cell population, (b) K 562 cell line, and (c) antibody-coated murine L 1210 tumor cells. Varying numbers (37.5×10^4 to 5×10^5) of T lymphocytes were incubated in V-bottomed wells of microtiter trays with 5×10^3 ^{51}Cr -labeled target cells at a final volume of 200 μl , thus ensuring lymphocyte/target cell ratios ranging from 7.5:1 to 100:1. The microplates were centrifuged at 100 g for 7 min. Cytolysis was then assessed by counting the radioactivity of 0.1 ml supernatant for 1 min in a scintillation counter. Specific lysis was calculated according to Cerottini et al. (13). Pokeweed mitogen (PWM) and tetanus toxoid stimulation were performed as previously described (11).

Results and Discussion

Unidirectional MLC were set up, to which BT3.4 (anti DC1) MAb was added at the onset of culture. It should be noted that either the responder or the stimulating cell population was DC1 positive. As BT3.4 is specific for that allele of the DC locus, this ensured that the antibody could react with the responder or the stimulating cell population, but not with both.

As shown in Table I, in no instance did the presence of the anti-DC1 Mab inhibit T cell proliferation. Under the same experimental conditions, an anti-DR Mab

TABLE I
Effect of Addition of Anti-DC1 (BT3.4) and Anti-DR (PTF29.12) Mab on Proliferative Response in MLC

Experiment Number	R	S	Antibody added	^3H]TdR cpm
1	DR 2,w6 DC1 ⁺	DR 5 DC1 ⁻	None	57,993 \pm 1,425*
			BT3.4	46,837 \pm 684
			PTF29.12	4,850 \pm 720
2	DR 2,w6 DC1 ⁺	DR 4,5 DC1 ⁻	None	52,325 \pm 3,198
			BT3.4	60,102 \pm 1,426
3	DR 1,2 DC1 ⁺	DR 5 DC1 ⁻	None	74,390 \pm 2,492
			BT3.4	67,853 \pm 2,880
4	DR 2,w6 DC1 ⁺	DR 3,7 DC1 ⁻	None	56,098 \pm 1,163
			BT3.4	51,918 \pm 1,022
5	DR 3,5 DC1 ⁻	DR 1,2 DC1 ⁺	None	58,344 \pm 2,802
			BT3.4	57,690 \pm 1,867
6	DR 5 DC1 ⁻	DR 2,w6 DC1 ⁺	None	45,933 \pm 2,518
			BT3.4	44,878 \pm 2,518
			PTF29.12	4,635 \pm 600

* Values are expressed as mean cpm \pm SD of triplicate cultures after having subtracted background counts due to responder and stimulator cells cultured alone.

reduced [^3H]TdR uptake to <10% the control value. Thus, unlike anti-DR antibodies, anti-DC1 does not interfere with T cell proliferation in MLC. Only one experiment is reported for the anti-DR MAb, as it reacts against a nonpolymorphic determinant of DR molecules and hence its effect is independent of the phenotype of the cell. Effector T cells are generated during MLC that are responsible for the lysis of (a) PHA-induced blast cells bearing the stimulating alloantigens, (b) K562 erythroid tumor line (nonspecific killing or NK-like), and (c) antibody-coated target cells (ADCC activity). We have investigated whether the anti-DC1 MAb had any effect on these various cytolytic activities. As shown in Figs. 1 and 2, when the effector cells were derived from MLC in which BT3.4 MAb was added from the onset of the culture, a marked difference in terms of specific cytolytic activity (CTL) was observed, depending upon the phenotype of the donors used. In fact, when the responding cells were DC1 negative and the stimulating cells DC1 positive (Fig. 1), no difference could be observed between the specific effector T cells generated in such combination and those obtained from control cultures to which BT3.4 was not added.

On the other hand, when the reverse combination was used (responder DC1 positive, stimulator DC1 negative), those activated T cells generated were virtually devoid of CTL activity. No differences were observed in between the combinations in terms of effector cells capable of killing in ADCC- or in NK-like activity (data not shown). Furthermore, the addition of BT3.4 directly to the cytolytic test could, at this stage, affect neither the specific cytolytic activity of DC1-positive effector T cells generated in MLC nor the killing of DC1-positive target cells (data not shown).

To check whether the anti-DC1 antibody had any effect on proliferation to a soluble antigen or on B cell differentiation, PBMC from DC1-positive donors were stimulated in culture with optimal concentrations of PWM or tetanus toxoid in the presence and in the absence of the MAb. Proliferation was evaluated by [^3H]TdR uptake, and B cell differentiation was determined by the number of cytoplasmic Ig (cIg) -positive cells stained by a fluorescein isothiocyanate (FITC) -coupled anti-

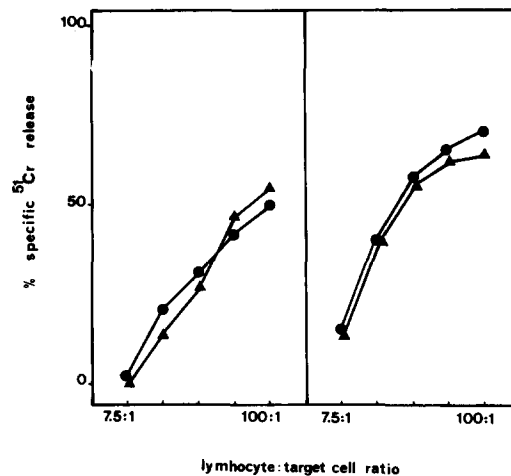


FIG. 1. Specific CTL activity of DC1-negative T cell populations activated in MLC. T cells were cultured for 7 d in allogeneic MLC, and thereafter their cytolytic activity against the PHA-activated cells bearing the stimulating alloantigens was tested. From left to right: experiments 5 and 6 of Table I. Untreated control (●); MLC treated with BT3/4 (▲).

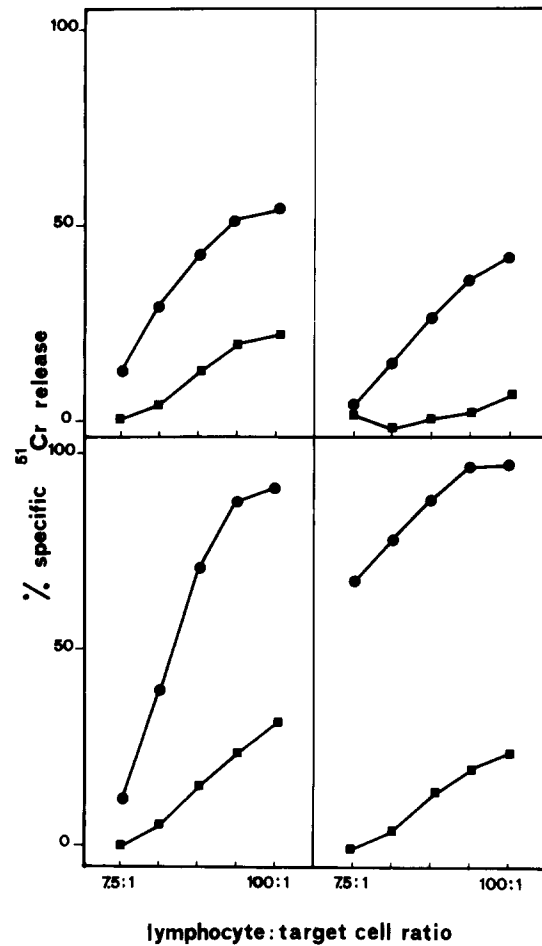


FIG. 2. Specific CTL activity of DC1-positive T cell populations activated in MLC. From top left to bottom right: experiments 1, 2, 3, and 4 of Table I. Untreated control (●); MLC treated with BT3/4 (▲).

human Ig antibody and the amount of Ig secreted in the culture fluid. As shown in Table II, the presence of BT3.4 reduced neither proliferation, the number of cIg positive cells, nor the amount of secreted Ig, whereas the anti-DR antibody abolished the response to both PWM and the soluble antigen (data not shown).

The above results show that in *in vitro* systems the effect of an antibody directed against a product of the DC locus is quite different from that of an antibody directed against a product of the DR locus. Thus, proliferation of T cells, or at least of most of them, is not disturbed by the anti-DC1 antibody, whereas unmasked DC molecules seem to be essential for proliferation and/or differentiation of CTL precursors in MLC. At this stage, however, it is impossible to tell whether these cells are blocked by a negative signal generated by the interaction of the antibody with membrane DC1 or because masking of the molecule prevents recognition of the allogeneic cell. Nevertheless, it is clear that DC molecules are neither receptors nor target molecules necessary for lysis of the target cells, as shown by the lack of effect of the antibody

TABLE II
*Effect of Addition of Anti-DC1 (BT3.4) and Anti-DR (PTF29.12) MAb
 on PWM and Tetanus Toxoid stimulation*

PWM		
Antibody added	cIg ⁺ cells/10 ⁶ cells	Secreted Ig
None	160,000	700 ± 107
BT3.4	115,000	630 ± 53
PTF29.12	25,000	150 ± 26
Tetanus Toxoid		
[³ H]TdR uptake*		
None	24,280 ± 1,561	
Bt3.4	22,630 ± 1,312	

* Values are expressed as mean cpm ± SD of triplicate cultures after having subtracted background counts due to PBMC cultured alone.

added directly to the cytolytic test. Taken together, the above results indicate that DC molecules are not only, as previously shown (6), structurally different from DR molecules, but also that they play distinct functions on the surface of B and T lymphocytes.

Summary

The products of the DC locus have been shown to be structurally different from those of the DR locus. In this paper it is shown that, unlike anti-DR antibodies, a monoclonal antibody directed against DC1 does not affect proliferation of T cells in response to alloantigens or soluble antigens or production of Ig in a pokeweed mitogen-stimulated in vitro culture. However, the anti-DC1 inhibits the generation of effector T cells mediating specific cytolytic activity, whereas no inhibitory effect can be observed on natural killer and antibody-dependent cytotoxic cell activities.

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