Brief Definitive Report

IL-2-MEDIATED T CELL PROLIFERATION IN HUMANS IS BLOCKED BY A MONOCLONAL ANTIBODY DIRECTED AGAINST MONOMORPHIC DETERMINANTS OF HLA-DR ANTIGENS

By ALLESANDRO MORETTA, ROBERTO S. ACCOLLA, AND JEAN-CHARLES CEROTTINI

From the Ludwig Institute for Cancer Research, Lausanne Branch, 1066 *Epalinges, Switzerland*

It has been well documented that human T cells that have been stimulated in unidirectional allogeneic mixed leukocyte cultures (MLC) may express surface antigens that are not detectable in resting T cells (1, 2). For example, HLA-DR (or Ia) antigens have been demonstrated on the surface of >60% of MLC T cells, whereas <6% of normal T cells have been found to be positive for these markers (3–5). Similarly, 4F2 antigen, a 120,000-mol wt protein normally found in peripheral blood monocytes (6), is not detectable in resting T cells, but is expressed in MLC stimulated T cells (7, 8).

Unlike resting T cells (9, 10), MLC-stimulated T cells can be propagated in vitro in the presence of interleukin 2 (IL-2) (11). In addition, IL-2 has been used to derive functional T cell clones (12–14). These clones express relatively large amounts of both Ia and 4F2 antigens irrespective of their functional activity (15).

In this study, we analyzed whether Ia and/or 4F2 antigens were involved in IL-2-dependent T cell proliferation. It will be shown that addition of D1/12 monoclonal antibody (Mab) directed against nonpolymorphic Ia determinants (16, 17) strongly inhibited IL-2 dependent proliferation of human MLC T cells and a cloned T cell line. In contrast, addition of anti-4F2 Mab had no effect on the proliferative response of human MLC or cloned T cells to IL-2. Moreover, addition of D1/12 Mab to murine MLC T cells, which do not express the determinants defined by D1/12 Mab, caused no inhibition of their proliferation induced by the same source of human IL-2.

Materials and Methods

Monoclonal Antibodies. Derivation of the two hybridoma antibodies (Mab) used in the present study has been described previously (16, 17). The anti-Ia D1/12 Mab is an IgG_2 antibody directed to nonpolymorphic determinants of human Ia molecules; 4F2 Mab is an IgG_2 antibody reacting against a 120,000-mol wt cell surface antigen expressed on peripheral blood monocytes and on activated T cells (7, 8). The two monoclonals were used under the form of purified IgG at a dose of 10 μg/well unless otherwise specified.

Indicator Cell System. Human MLC T cells were obtained from unidirectional 7-d MLC containing 10^6 responding peripheral blood lymphocytes and 10^6 irradiated (5,000 rad) allogeneic cells as described previously (18). T cells were purified according to their capacity to form spontaneous rosettes with sheep erythrocytes and stored frozen in liquid nitrogen until used. Clone 16 is a human T cell clone obtained from MLC T cells by limiting dilution

techniques. The growth of clone 16 cells is dependent upon the presence of exogenous IL-2 in the culture medium. The cells grow without the presence of feeder cells, do not express Fc receptors for IgG, but express both Ia and 4F2 antigens. The functional properties as well as the surface phenotype of clone 16 cells have been described in a previous report (15). Long-term MLC murine cells were obtained as described elsewhere (19) from 14-d MLC cultures containing C57BL/6 responding spleen cells and irradiated DBA/2 stimulating spleen cells.

Source of IL-2. Supernatants containing IL-2 activity were obtained from cultures of human spleen cells stimulated with phytohemagglutin (PHA) as described in detail elsewhere (20). Supernatants were depleted of PHA by two-step sequential precipitation with 40 and 80%-saturated ammonium sulphate (SAS) as previously described (21). The 80% SAS-precipitated material was dialyzed and resuspended in a volume of RPMI 1640 medium corresponding to one half of the original culture volume.

Assay for IL-2 Activity. 10^4 indicator cells (human or mouse MLC cells and clone 16 cells) were cultured for 72 h in RPMI 1640 medium (Gibco Bio-Ag, Basel Switzerland) supplemented with 10% fetal calf serum FCS (Gibco Bio-Ag) in round-bottomed microtiter trays (Greiner Labor Tecknik, Nurtingen, Germany). IL-2-containing medium was added at a final dilution of 1:2 (total volume 200 μl). [^{3}H]thymidine (TdR) was added (0.1 μCi/well, sp act 9 mCi/mM) 6 h before harvesting the cultures. The uptake was determined according to Greaves et al. (22). The counts per minute reported represent the mean value of triplicate cultures.

Results and Discussions

In a first series of experiments, 10^4 human MLC T cells were cultured with IL-2-containing medium in the presence or absence of D1/12 or 4F2 hybridoma culture supernatant (final dilution, 1:20). Table I displays the results of three representative experiments. In the presence of D1/12 Mab, IL-2-induced proliferation was markedly inhibited. Dose-response analysis showed that >90% inhibition of proliferation was observed in cultures containing D1/12 hybridoma supernatant diluted up to 1:160 (corresponding to ~1.25 μg antibody IgG) (Fig. 1). In contrast, no inhibition of proliferation was found in the presence of a 1:20 dilution of either 4F2 hybridoma or

TABLE I

Effect of D1/12 Mab on the Proliferative Capacity of MLC T Cells in Response to IL-2

Experiment	IL-2* added	Mab added‡	[^{3}H]TdR uptake§
			cpm
1	−	None	1626 ± 304
	+	None	28342 ± 789
	+	D1/12	4828 ± 377
	+	4F2	26431 ± 1722
	+	PX63	29004 ± 2421
2	−	None	984 ± 92
	+	None	35155 ± 2644
	+	D1/12	3831 ± 182
	+	4F2	32480 ± 1922
	+	PX63	33631 ± 2862
3	−	None	633 ± 46
	+	None	21902 ± 1561
	+	D1/12	2346 ± 255
	+	4F2	20686 ± 1044
	+	PX63	19799 ± 2643

* 100 μl of IL-2-containing medium (see Materials and Methods) was added to 100 μl of culture medium containing 10^4 indicator cells.

‡ 10 μg of purified IgG of the different Mab listed were added at the onset of the culture.

§ After 3 d of culture, cells were pulsed with [^{3}H]TdR for 4 h. Values are expressed as mean counts per minute ± standard deviation of triplicate cultures.

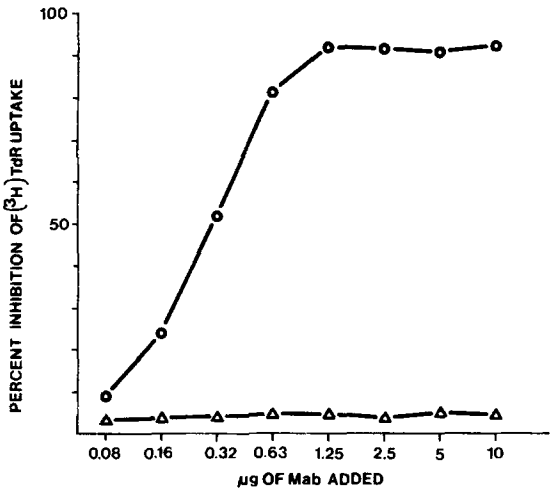

FIG. 1. Effect of addition of different amounts of D1/12 or 4F2 Mab on the proliferative capacity of human MLC-T cells in response to human IL-2. Triplicate microwell cultures of 1×10^4 primary MLC T cells were set up in RPMI medium with 10% FCS, 50% vol/vol of IL-2-containing medium and the amount of specific Mab listed in the abscissa (total culture volume: 200 μl). After 3 d of culture, cell proliferation was evaluated by adding 0.1 μCi/well of [^{3}H]TdR as described in Materials and Methods. O, D1/12; Δ, 4F2. Abscissa, amount of Mab added in μg of purified IgG; ordinate, percent inhibition of [^{3}H]TdR uptake as compared to control cultures in which the addition of Mab was omitted.

parent myeloma PX63 culture supernatant (Table I). Similar results were obtained in nine additional experiments involving different responder-stimulator cell combinations.

Given (*a*) the ability of MLC T cell populations to mediate antibody-dependent lysis (23, 24), and (*b*) the large proportion of MLC T cells bearing Ia antigens, these results could have been explained by the killing of D1/12 Mab-coated cells by effector cells present in the MLC populations. To rule out this possibility, we tested the effect of D1/12 Mab on Il-2-induced proliferation of a cloned T cell line. As shown previously (15), clone 16 cells do not bear Fc receptors for IgG, nor do they mediate antibody-dependent lysis. As assessed by flow cytofluorometry, Ia and 4F2 antigens are expressed in similar amounts on the surface of clone 16 cells (data not shown). As shown in Table II, the proliferation of clone 16 cells in the presence of IL-2 was markedly inhibited in cultures containing D1/12 Mab, but was unaffected in cultures containing 4F2 Mab or PX63 culture supernatant.

Because these results indicated that inhibition of proliferation by D1/12 Mab was not caused by antibody-dependent cell-mediated lysis of Ia$^+$ T cells, we then considered the possibility that D1/12 Mab could functionally inactivate the human IL-2 preparation used in this study. If this was the case, the inhibitory effect of D1/12 Mab on IL-2-induced proliferation would be exerted independently of the presence of Ia antigens on IL-2-responding T cell populations.

Because human IL-2 has been shown to be active on murine T cells (25), we used long-term C57BL/6 anti-DBA/2 MLC cells (19) as a source of IL-2-responding T cells. These cells, after 3 d of culture in the presence of human IL-2, exhibit a strong proliferative response upon reexposure to human IL-2 (Table III). However, they do

TABLE II

Effect of D1/12 Mab on the Proliferative Capacity of Clone 16 Cells in Response to IL-2

Experiment	IL-2* added	Mab added‡	^{3}HTdR uptake§
			cpm
1	−	None	564 ± 188
	+	None	61204 ± 7382
	+	D1/12	7342 ± 1077
	+	4F2	64826 ± 9881
	+	PX63	60300 ± 4223
2	−	None	301 ± 82
	+	None	42877 ± 3566
	+	D1/12	3919 ± 655
	+	4F2	40872 ± 2993
	+	PX63	41624 ± 2773
3	−	None	634 ± 128
	+	None	33622 ± 926
	+	D1/12	2977 ± 645
	+	4F2	30428 ± 1076
	+	PX63	31977 ± 2322

* As in Table I.
‡ As in Table I.
§ As in Table I.

TABLE III

Addition of D1/12 Mab Does Not Inhibit the Proliferation Response of Murine MLC Cells to Human IL-2

Experiment	IL-2* added	Mab added‡	[^{3}H]TdR uptake§
			cpm
1	−	None	328 ± 73
	+	None	18831 ± 344
	+	D1/12	17446 ± 478
	+	4F2	17881 ± 422
2	−	None	252 ± 61
	+	None	23287 ± 1941
	+	D1/12	21844 ± 2001
	+	4F2	23064 ± 3614
3	−	None	227 ± 40
	+	None	16871 ± 918
	+	D1/12	15990 ± 333
	+	4F2	15443 ± 722

* 100 μl of human IL-2-containing medium was added to 100 μl of culture medium containing 10^4 murine MLC cells derived from C57BL/6 anti-DBA/2 long-term MLC as described previously (19).
‡ As in Table I.
§ As in Table I.

not react with D1/12 Mab as assessed by quantitative immunofluorescence and complement-dependent lysis (data not shown). Table III shows that addition of D1/12 Mab to cultures of murine MLC cells had no effect on human IL-2-induced proliferation.

Further evidence that D1/12 Mab did not react with human IL-2 was provided by the demonstration that IL-2-containing supernatants that had been twice passed through an immunoadsorbent column of D1/12 Mab coupled-Sepharose beads showed no decrease in IL-2 activity (data not shown).

These experiments clearly indicate that the inhibition of IL-2-induced proliferation of human MLC-T cells is a consequence of a direct interaction of D1/12 Mab with surface Ia molecules. The precise mechanism(s) by which anti-Ia Mab inhibits T cell proliferation has still to be determined. Several possibilities can be envisaged: (*a*) Anti-Ia Mab may inhibit the binding of IL-2 to the corresponding surface receptors by steric hindrance. Previous studies have shown that D1/12 Mab reacts with a specific subset of human Ia antigens accounting for only a portion of the human Ia molecular pool (16, 17). Therefore, if the above mentioned mechanism is correct, one should envisage a topographic close association between IL-2 receptors and this limited portion of Ia molecules. (*b*) Ia molecules may participate in the cellular events required for the transmission of the signal(s) given by IL-2 and resulting in cell proliferation. Studies are in progress in our laboratory to discriminate between these or other possibilities.

Summary

We have studied the effect on the interleukin (IL-) 2-dependent human T cell growth of two distinct monoclonal antibodies (Mab), D1-12 and 4F2, with specificity for common determinant of human Ia antigens and for a differentiation antigen expressed on all activated T cells, respectively. Strong inhibition of cell growth was found in cultures supplemented with the anti-Ia D1-12 Mab but not in cultures supplemented with 4F2 Mab. These results were obtained when either total mixed leukocyte culture (MLC) T cells or an MLC-derived T cell clone were used as indicator cell systems for IL-2 activity.

The inhibition of cell growth appears to be mediated by a direct interaction of D1-12 Mab with the cells and not by a direct inactivation of the growth factor, as addition of the antibody to murine MLC T cells, which do not express the determinant defined by D1-12 Mab, resulted in no inhibition of their proliferation induced by the same source of human IL-2.

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