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Monoclonal antibodies against a rat leucocyte antigen block antigen-induced T-cell responses via an effect on accessory cells

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SUMMARY

The MRC OX-45 and OX-46 mouse monoclonal antibodies recognize a rat cell surface glycoprotein of 45,000 MW that is present on a wide variety of haematopoietic cells and on endothelial cells. MRC OX-45 IgG or $F(ab')_2$ blocked the primary mixed lympocyte response (MLR) and the secondary response of T lymphocytes to the soluble antigen DNP–BGG. In contrast, the antibodies had no effect on the cytotoxic activity of specific (CTL) or non-specific (NK) killer cells or on proliferative responses stimulated by lectins or oxidative mitogenesis. The inhibitory effect was at the level of stimulator cells rather than responders since mouse anti-rat xenogeneic MLRs were inhibited but rat anti-mouse responses were unaffected. However, the effect was not a direct one because inhibition was seen when irradiated spleen cells were used as stimulators but not when cell populations highly enriched for dendritic cells were used. In the latter case, inhibition potentiated by antibody could be restored if a peritoneal cell population enriched for macrophages was added back to the cultures. The inhibitory effects of these monoclonal antibodies seem most likely to be due to potentiation of non-specific suppression by macrophages.

INTRODUCTION

One approach to determining the functional relevance of cell surface molecules is to test for effects of monoclonal antibodies (MAbs) on various cellular activities (Webb, Mason & Williams, 1979). MAbs that react to the CD3, CD4, CD8, CDW18 and class II MHC molecules inhibit various proliferative and cytotoxic responses of T cells by binding to the responding T cells or to the cells with which they are reacting (Townsend, 1985). We have used the rat allogeneic MLR between responder lymph node cells and stimulator spleen cells as a screening test to identify new molecules worthy of further analysis. This paper describes two MAbs, designated MRC OX-45 and OX-46, that react with the same antigen and block T-cell proliferative responses to alloantigens. However, the inhibitory activity of these MAbs did not result from binding to either the responder T_H cells or the allogeneic dendritic cells that are responsible for stimulating cell division in the MLR. Instead, we show that the inhibitory effect is mediated via accessory cells that may be macrophages.

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Abbreviations: BSA, bovine serum albumin; Con A, concanavalin A; DAB, Dulbecco's A+B medium; DNP–BGG, dinitrophenylated bovine γ-globulin; Ig, immunoglobulin; IL-2, interleukin-2; MAb(s), monoclonal antibody(ies); MLR, mixed lymphocyte reaction; PBS, phosphate-buffered saline; PHA, phytohaemagglutinin; T_H, T helper; TDL, thoracic duct lymphocytes.

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MATERIALS AND METHODS

Inbred rat strains PVG. $RT1^c$, PVG.$RT1^u$ (AO), and DA.$RT1^a$ (referred to as HO, HO.B2 and DA, respectively) and F_1 hybrids HO × HO.B2 were obtained from the Specific Pathogen-Free Unit of the MRC Cellular Immunology Unit, Oxford. (DBA/2 × BALB/c)F_1 mice were bred from parent strains obtained from Olac (1976) Ltd (Bicester, Oxon).

Antibodies

The mouse anti-rat monoclonal antibodies MRC OX-45 (IgG1) and MRC OX-46 (IgG2a) were produced by immunizing BALB/c mice with rat T blasts prepared by stimulating purified T helper (T_H) cells with allogeneic irradiated rat spleen cells (Mason, Pugh & Webb, 1981) and fusing the mouse spleen cells with the NSO/1 myeloma cell line using routine procedures (Galfre & Milstein, 1981).

MRC OX-45 and OX-46 IgG were purified from ascites fluid, and $F(ab')_2$ fragments were prepared from MRC OX-45 IgG by digestion with 2% (w/w) pepsin in 0·1 M sodium acetate (pH 4) followed by gel filtration on a Sephacryl S200 column (Mason & Williams, 1980).

Other MAbs used were OX-1 (anti-rat L-CA), OX-6 (anti-rat class II MHC), OX-8 (anti-rat CD8), OX-12 (anti-rat kappa

chain) (see Mason *et al.*, 1983), and OX-39 [anti-rat IL-2 receptor–proven on the basis that IL-2 binding to rat T blasts is inhibited by the antibody (D. Paterson, P. Cortese, J. Green, W. Jefferies, M. Puklavec and A. Williams, manuscript in preparation)].

Cells

Cervical lymph nodes and spleen were removed aseptically and teased apart in ice-cold Dulbecco's A+B medium (DAB) containing 0·2% bovine serum albumin (BSA). The cells were filtered through cotton wool, washed twice in DAB/BSA and resuspended in RPMI-1640 medium containing $2{\cdot}5 \times 10^{-5}$ M 2-mercaptoethanol and antibiotics.

T helper (T_H) cells were prepared from thoracic duct lymphocytes (TDL) by removing CD8 (OX-8)-positive $T_{cytotoxic/suppressor}$ cells and MRC OX-12-positive B cells using rosette depletion (Mason *et al.*, 1981).

Dendritic cells, in the thoracic duct lymph of rats that had been mesenteric lymphadenectomized, were purified by centrifugation at 600 *g* for 10 min on 15% (w/v) metrizamide gradient (Nyegaard, Oslo) (Mason *et al.*, 1981).

Resident peritoneal cells were obtained by washing the peritoneal cavity with 15 ml PBS/0·2% BSA. To enrich for macrophages, the cell suspension was layered over 2 ml of 20% (w/v) metrizamide in PBS/0·2% BSA and centrifuged for 10 min at 600 *g* at 4°. Fifty percent of the starting cells were recovered at the interface and, of these >90% were macrophages as judged by morphology and latex particle ingestion. Peritoneal macrophages and the MLR stimulators (dendritic cells or spleen cells) were given 1500 rads of γ-irradiation from a ^{137}Cs source at 100 rads/min.

Functional assays

The origins and doses of cells used for assaying lymphocyte proliferative responses to mitogens and allogeneic or xenogeneic cells are given in the legends to figures. Cells were cultured in a total volume of 0·2 ml/well RPMI+5% DA serum in sterile 96 U-well microtitre plates (Titertek 76.013.05, Flow Laboratories, Irvine, Ayrshire) at 37° in a humidified incubator with 5% CO_2. The cultures were pulsed with 0·5 μCi [6-^{3}H]thymidine (Amersham International, Amersham, Bucks) per well 18–24 hr before the end of the incubation period, and uptake of radioactivity was determined after harvesting onto glass-fibre filters (Whatman, Maidstone, Kent). T-cell mitogenesis induced by sodium periodate (oxidative mitogenesis) was as described in Austyn *et al.* (1983). Secondary responses to a soluble antigen were studied by priming HO.B2 rats with an intraperitoneal injection of 0·5 mg of alum-precipitated–dinitrophenylated bovine γ-globulin (DNP–BGG) mixed with pertussis vaccine. Four weeks later, 5×10^5 spleen cells were cultured with the challenge antigen at 10–50 ng/ml for a total of 7 days. Antibody secretion was then assayed on the supernatants using a soft plate coated with DNP–BGG and ^{125}I F(ab′)$_2$ anti-rat kappa chain (OX-12) as a second antibody. T-cell (CTL) and natural killer (NK) cytolysis assays were as described by Green (1984). IL-2 activity in cell culture supernatants was assayed by adding the supernatant at dilutions of 1:2, 1:4 and 1:8 to 4×10^4 T blasts and measuring proliferation after 24 hr as previously described. The T blasts were prepared by culturing HO spleen cells at 2×10^6 cells/ml with 5 μg/ml Con A for 72 hr and then washing in 20 mg/ml of methyl-α-D-mannoside. A crude source of IL-2 was also obtained as a lectin-free supernatant from Con A-activated rat spleen cells (Wilson *et al.*, 1982).

RESULTS

Effects of MRC OX-45 and OX-46 antibodies on *in vitro* functional assays

MRC OX-45 and OX-46 are distinct MAbs raised against activated rat T lymphocytes that react with the same antigen in a manner such that one antibody blocks the binding of the other. The antigen recognized is a 45,000 MW glycoprotein expressed on all haematopoietic cells and endothelium, and these aspects will be described elsewhere (Arvieux, Willis & Williams, 1986). When either antibody was added at the start of a primary MLR between HO or HO.B2 lymph node cells and F_1 (HO × HO.B2) spleen cells, lymphocyte proliferation was strongly inhibited. Results for [^{3}H]thymidine incorporation are shown in Fig. 1, and proliferation as assessed by the production of blast cells was also inhibited (not shown). MRC OX-45 F(ab′)$_2$ fragments showed the same dose–response curve for inhibition as the IgG. The plateau levels of inhibition averaged 82±12% SD in seven independent experiments using antibody concentrations up to 20 μg/ml. The inhibitory activity was not attributable to cytotoxicity as determined by trypan blue dye exclusion.

The effect of OX-45 IgG on the secondary response of primed spleen cells to an *in vitro* challenge with DNP–BGG was assayed by cell proliferation and anti-DNP antibody secretion. Both of these responses were blocked by adding OX-45 IgG to cultures and the data for the proliferation assay is shown in Table 1 (Experiment A).

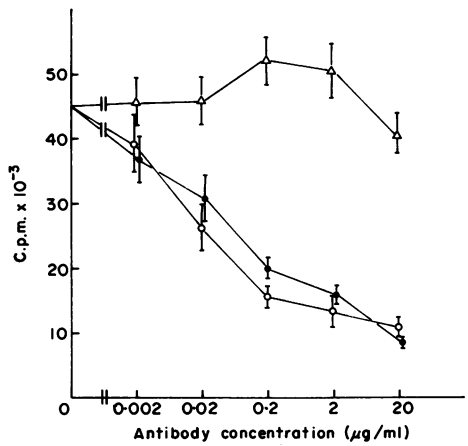

Figure 1. Effect of MRC OX-45 and MRC OX-46 MAbs on MLR proliferation. HO lymph node cells ($2{\cdot}5 \times 10^5$) were incubated with 5×10^5 irradiated F_1 spleen cells for 4 days in RPMI medium containing 5% DA serum. The cells were cultured in 0·2 ml/well in sterile microtitre plates. Monoclonal antibodies were added to the culture at the beginning of the incubation as pure IgG and/or F(ab′)$_2$ for MRC OX-45 antibody. Cultures were labelled with 0·5 μCi [^{3}H]thymidine 18 hr before the end of the incubation period, and the cells were harvested on glass-fibre filters. Data are means and ranges of quadruplicate determinations. Cells were cultured with MRC OX-46 IgG (●) MRC OX-45 F(ab′)$_2$ (○) or MRC OX-1 IgG included as a control (△). The dose–response curve for MRC OX-45 IgG (not shown) was similar to those for MRC OX-46 IgG and MRC OX-45 F(ab′)$_2$.

Table 1. Effect of MRC OX-45 on T-cell proliferative responses to various stimulants

Exp.	Stimulant	Responder cells	[^{3}H]thymidine incorporation (c.p.m. $\times 10^{-3}$) Unstimulated background	Stimulated cultures	Stimulated + MRC OX-45 IgG
A	DNP–BGG (50 ng/ml)	5×10^5 primed SC*	3·2±0·7	14·4±3·3	2·1±0·2 (0)
B	5×10^5 allogeneic SC	$2{\cdot}5 \times 10^5$ LNC†	0·9±0·2	30·2±9·7	1·4±0·3 (0)
	5×10^5 allogeneic SC	$2{\cdot}5 \times 10^5$ LNC			6·2±1·9 (1)
	5×10^5 allogeneic SC	$2{\cdot}5 \times 10^5$ LNC			11·2±2·4 (2)
	5×10^5 allogeneic SC	$2{\cdot}5 \times 10^5$ LNC			11·0±1·5 (3)
	5×10^5 allogeneic SC + Con A supernatant	$2{\cdot}5 \times 10^5$ LNC		93·6±4·2 (0)‡	108·4±4·9 (0)
				52·9±8·4 (1)	3·2±1·7 (0)
				36·9±2·4 (2)	1·5±0·4 (0)
				26·4±3·1 (3)	1·3±0·9 (0)
C	Syngeneic irradiated SC	$2{\cdot}5 \times 10^5$ periodate-treated LNC	1·5±0·3	67·5±5·0	67·0±6·0 (0)
D	PHA (2 μg/ml)	5×10^5 SC	1·2±0·4	25·2±5·5	24·5±2·6 (0)
E	Con A (4 μg/ml)	5×10^5 SC	1·8±0·3	16·3±1·8	16·1±1·4 (0)

Data are means ± standard deviation of 0·2 ml quadruplicate cultures in RPMI + 5% DA serum incubated for 24 hr (C), 48 hr (D) 72 hr (B and E) or 96 hr (A) with the indicated stimulant in the presence or absence of MRC OX-45 IgG at 20 μg/ml. Proliferation was then assayed by an 18-hr [^{3}H]thymidine pulse.

* SC, spleen cells.

† LNC, lymph node cells.

‡ The numbers in parentheses after figures in the last two columns indicate the period of culture in days before which Con A supernatants (second last column) or MAbs (last column) were added.

In contrast to the effects on antigen-stimulated proliferation, the antibodies did not block proliferation induced by Con A or PHA lectins (Table 1, Experiments D and E) or by oxidative mitogenesis (Table 1, Experiment C). In addition, the antibodies had no effect on cytolysis due to specific T cells generated in a primary MLR or to natural killer cells derived from macrophage-depleted spleen (data not shown).

Parameters of inhibition of the MLR

The antibodies might have blocked proliferation induced by alloantigens by affecting the level of IL-2 production and/or by producing a lag in the kinetics of response. A time–course analysis of IL-2 production was done to test these possibilities. A primary MLR between HO lymph node cells and (HO × HO.B2) F_1 spleen cells was set up with or without MRC OX-45 antibody, and the supernatants from these cultures were harvested daily for 5 consecutive days, then assayed for IL-2 activity. The results show (Fig. 2) that the level of IL-2 production is reduced in the presence of MRC OX-45 IgG by a maximum of 57% at the peak of the response, and that this inhibitory effect is maintained throughout the 5-day culture period. Neither MRC OX-45 nor MRC OX-46 IgG was able to inhibit proliferation of activated T cells induced by IL-2 (not shown). In order to test whether MRC OX-45 inhibits an early event in the MLR, the antibody was added at the initiation of the culture or at various times thereafter (24, 48 or 72 hr). As shown in Table 1 (Experiment B), MRC OX-45 significantly inhibited the MLR, even when it was added to the cultures 3 days after their initiation. In order to determine whether the level of IL-2 was actually the limiting factor, we examined the ability of conditioned medium from Con A-stimulated rat spleen cells to restore proliferation in MLR cultures inhibited by MRC OX-45 IgG. The results show (Table 1, Experiment B) that crude IL-2 supernatant added at the initiation of the MLR

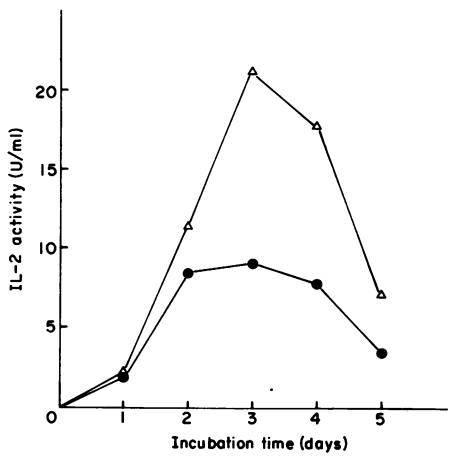

Figure 2. Time–course of IL-2 production in MLR in the presence of MRC OX-45 IgG. Primary MLR between HO lymph node cells ($2{\cdot}5 \times 10^5$) and (HO × HO.B2) F_1 spleen cells (5×10^5) were incubated for 5 days in RPMI containing 5% DA serum with (●) or without (△) MRC OX-45 IgG added at the beginning of the culture. Supernatants were collected daily and IL-2 activity was assayed on Con A-activated blasts. One U/ml of IL-2 activity corresponded to the dilution that produced 10% maximal proliferation of target cells.

increased proliferation of MRC OX-45-inhibited cultures to levels equal to or greater than that obtained in control MLR plus IL-2-containing supernatant. However, this effect was probably not due simply to the IL-2 in the supernatant because addition at later times did not overcome the inhibition by antibody (Table 1, Experiment B).

In order to investigate whether MRC OX-45 antibody caused an impairment in IL-2 receptor expression on alloantigen-activated T cells, we used ^{125}I-OX-39 IgG, a mouse MAb that recognizes the rat IL-2 receptor. The cell pellets from primary MLR cultures treated or untreated with MRC OX-45 IgG bound similar numbers of OX-39 molecules (2666 ± 148 and 2418 ± 293 c.p.m., respectively, for an unstimulated background of 832 ± 140 c.p.m. with lymph node cells and spleen cells mixed in the binding assay). Parallel cultures showed that MRC OX-45 IgG had inhibited lymphocyte proliferation (97%) in the same experiment.

Cells affected by the antibodies

Since the MRC OX-45 and OX-46 MAbs react with various cell types, including T cells, dendritic cells and macrophages, it was important to establish the identity of the target cells mediating the suppressive effects of the antibodies. This was studied in a xenogeneic MLR between rat and mouse using (BALB/c × DBA/2)F_1 mouse spleen cells (which do not bind MRC OX-45 antibody) either as stimulators with rat lymph node responders or as responders with rat spleen stimulators. A representative experiment shown in Table 2 demonstrates that the inclusion of MRC OX-45 IgG fails to inhibit proliferation in the rat versus mouse combination, while it almost completely blocks the mouse against rat response and, as in the allogeneic MLR, produces about 60% inhibition when added 72 hr after the beginning of the cultures. Thus, the antibody seems to be affecting cells in the stimulator population rather than the responder T cells.

Because, under our experimental conditions, dendritic cells are the major, if not exclusive, source of stimulator cells, it was possible that the MAbs were acting at the level of these cells. However, results in Fig. 3a show that OX-45 MAb failed to inhibit an allogeneic MLR using a fixed number of HO TDL and titrated doses of irradiated dendritic cells (purified from thoracic duct lymph of HO.B2 rats following mesenteric lymphadenectomy) as stimulators.

The possibility was thus examined that the MLR was inhibited because of an interaction between OX-45 antibody and other accessory cells present in the spleen population. Macrophages are a candidate for such inhibition, since high numbers of macrophages have been reported to have an inhibitory effect on lymphocyte proliferation (Allison, 1978; Ju & Dorf, 1985). Therefore, peritoneal macrophages (>90% pure) or whole spleen cells were titrated for suppressive activity against an MLR between TDL and dendritic cells in the presence or absence of OX-45 IgG (Fig. 3b and c). Peritoneal macrophages and spleen cells of responder or stimulator strain were used with the same results. The spontaneous suppressive activity of the peritoneal cells was particularly noticeable (Fig. 3b) with complete inhibition at a ratio of 1:10, peritoneal cells:TDL, and the antibody potentiated the effect by a factor of two. With the addition of spleen cells, the suppressive effect without antibody was seen only at the highest cell dose, but the antibody again potentiated suppression at lower doses (Fig. 3c). Similar results were obtained in this experiment by using a responder population of purified T_H cells. These cells had been depleted of the CD8 (OX-8)-positive T subset, and thus any suppressor cells that might be in this subset are not secondarily involved to effect suppression.

In order to determine whether the secretion of prostaglandins accounted for the inhibition of proliferative responses, MLR cultures as above were set up in the presence of indomethacin, a potent inhibitor of prostaglandin synthesis. The addition of indomethacin in concentrations up to 10^{-5} M did not alter the spontaneous or MAb-induced suppressive activity of the peritoneal macrophages or spleen cells on

Table 2. Effect of MRC OX-45 IgG on xenogeneic MLRs between rat and mouse

		[^{3}H]thymidine incorporation (c.p.m. × 10^{-3})			
Responders	Irradiated stimulators	Unstimulated background*	Control MLR	MLR + MRC OX-45 IgG (day antibody added)	% inhibition
$2{\cdot}5 \times 10^5$ rat LNC (HO)	5×10^5 mouse SC (BALB/c × DBA/2) F_1	$1{\cdot}5 \pm 0{\cdot}3$	$152{\cdot}3 \pm 18$	$154{\cdot}5 \pm 11$	0
5×10^5 mouse SC (BALB/c × DBA/2) F_1	3×10^5 rat SC (HO)	$5{\cdot}8 \pm 1{\cdot}1$	$27{\cdot}5 \pm 3{\cdot}2$	$9{\cdot}5 \pm 2{\cdot}6$ (0)	83
				$14{\cdot}2 \pm 1{\cdot}7$ (1)	62
				$15{\cdot}2 \pm 2{\cdot}4$ (2)	57
				$14{\cdot}8 \pm 3{\cdot}2$ (3)	59

(BALB/c × DBA/2) F_1 mouse spleen cells (SC) that do not express MRC OX-45 antigen were used either as stimulators with HO lymph node (LNC) responders or as responders with HO spleen stimulators in xenogeneic MLR. Cultures in RPMI + 5% DA serum were incubated for 3 days with or without MRC OX-45 IgG (20 μg/ml) added at various times as indicated. Proliferation was assayed by a 18-hr [^{3}H]thymidine pulse. Data are means ± standard deviation of quadruplicate cultures from one representative experiment.

* Cultures without irradiated stimulator cells.

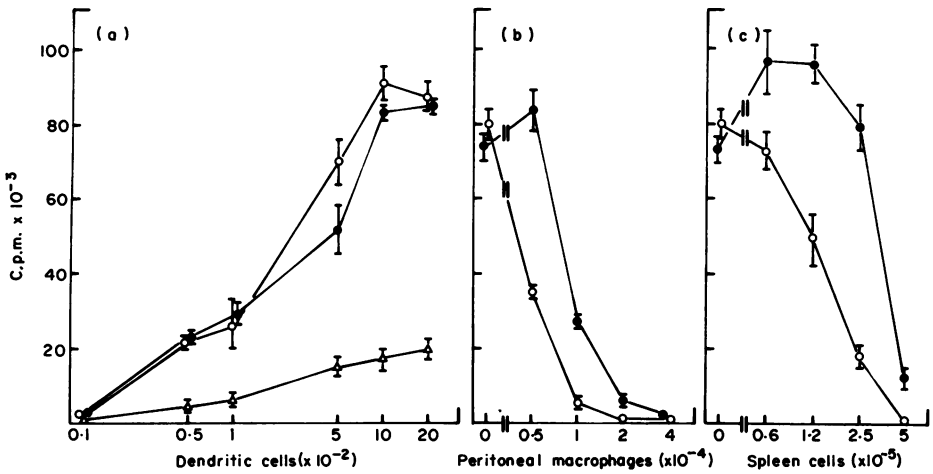

Figure 3. Requirement for accessory cells to effect suppression of MLR through MRC OX-45 MAb binding. An MLR was set up between purified HO.B2 dendritic cells, titrated (a) or used at the optimal concentration of 10^3 stimulator cells/well (b and c) and $2{\cdot}5 \times 10^5$ HO thoracic duct lymphocyte responders either without (a) or with the addition of various numbers of irradiated HO peritoneal cells enriched for macrophages (b) or spleen cells (c) as indicated. Quadruplicate 0·2 ml cultures in RPMI + 5% DA serum were incubated for 3 days in the absence (●) or in the presence of MRC OX-45 IgG at 20 μg/ml (○) before proliferation was assayed as previously described. MRC OX-6 IgG, an anti-Ia MAb, was included at 20 μg/ml in (a) (△) to establish that the cultures could be inhibited by an antibody that should show this effect. MRC OX-1 IgG was used as a negative control in (b) and (c) and produced no inhibition (not shown).

Table 3. Indomethacin does not reverse suppression mediated by peritoneal cells

TDL × DC (HO) (HO.B2) cocultured with:				
Peritoneal cells (HO) (10^4)	OX.45 IgG (μg/ml)	IM (μM)	[^{3}H]thymidine incorporation (c.p.m. × 10^{-3})	% inhibition
—	—	—	91·7 ± 15·7	—
—	20	—	92·6 ± 4·7	0
—	—	10	118·3 ± 20·9	0
1	—	—	44·8 ± 1·4	51
1	20	—	22·8 ± 3·3	75
1	—	10	43·6 ± 14	63
1	20	10	15·2 ± 5·7	84

An allogeneic MLR was set up between $2{\cdot}5 \times 10^5$ thoracic duct lymphocytes (TDL) and 10^3 dendritic cells (DC), and cocultured with peritoneal cells and MRC OX-45 IgG in the presence or absence of 10 μM indomethacin (IM). Proliferation was assessed by a 24-hr [^{3}H]thymidine pulse given after 72 hr of culture. Unstimulated TDL incorporated 1067 c.p.m. Data are means ± standard deviation of quadruplicate cultures.

lymphocyte proliferation (Table 3). Furthermore, even in the absence of indomethacin, the cell-free supernatants harvested on Day 3 from inhibited cultures (containing peritoneal cells and MRC OX-45 IgG) lacked inhibitory activity when combined with fresh MLR cultures between TDL and dendritic cells (data not shown—the antibody in these supernatants does not by itself inhibit cultures stimulated by dendritic cells).

DISCUSSION

The MRC OX-45 and OX-46 antibodies were of interest because they showed inhibition of the allogeneic MLR using lymph node cells as responders and spleen cells as stimulators. This inhibition appeared to be due to an effect on the stimulating cell population because a xenogeneic MLR in which mouse T cells responded to rat spleen cells was blocked whereas the rat against mouse response was not affected. However, the MAbs were not likely to be acting directly on the dendritic cells that stimulate cell division in the MLR (Mason *et al.*, 1981; Van Vorrhis *et al.*, 1983; Klinkert, LaBadie & Bowers, 1982) because the OX-45 MAb had no effect on an MLR between TDL and purified dendritic cells, whereas the addition of macrophage-enriched peritoneal cells or unfractionated spleen cells to these cultures restored the inhibition mediated by OX-45 MAb. The most likely target for the inhibitory effect of the antibodies appeared to be macrophages, but it cannot be ruled out that a minor cell population is responsible.

Mouse suppressor macrophages have been identified in spleen from neonates (Piguet, Irle & Vassalli, 1981) or after total lymphoid irradiation in adults (May, Slavin & Vitetta, 1983) or after *in vitro* culture of spleen cells for two or more days (Stout & Fisher, 1983a; Veit, 1982). The appearance of similar inhibitory macrophages *in vitro* has been found to be associated with chronic perturbations of the immune system (reviewed in Stout & Fisher, 1983a), including cancer, sarcoidosis, and some autoimmune and infectious diseases. The mechanisms by which macrophages become suppressive and produce suppression appear diverse, and the following might be considered. (i) Macrophages suppress by killing the responder T cells (Rao *et al.*, 1983); unlikely in this case since mitogenic responses were not affected by addition of OX-45 antibody. (ii) T suppressor cells are somehow implicated (Beckwith & Rich, 1983); this would not be so for CD8 (OX-8)-positive suppressors, but suppression via CD4 (W3/25)-positive T cells (Hall *et al.*, 1985)

not ruled out. (iii) Suppression may occur by agglutination of T responders with stimulators; not likely since the response of mouse T cells (not labelled by the MAbs) to rat stimulators was inhibited. (iv) Soluble factors from macrophages may be involved, including thymidine, prostaglandins, oxygen metabolites, complement components, interferon, arginase and macrophages suppressor factor [reviewed in Allison (1978)]. The first three of these seem unlikely; thymidine would effect only [^{3}H]thymidine uptake (Stadecker *et al.*, 1977) but production of blast cells was also inhibited; prostaglandin production should be inhibited by indomethacin but this did not alleviate the effect of the MAbs; effects of oxygen metabolites should be prevented by the 2-mercaptoethanol in the culture. Furthermore, there was no evidence for inhibition by any soluble factor since supernatants from inhibited cultures did not affect other cultures. There was the possibility that the inhibitory effect was mediated via an inhibition of IL-2 production since the level of IL-2 in the MAb-inhibited cultures was about half that in control cultures, and addition of Con A supernatant on Day 0 reversed the inhibition of proliferation. However, if the supernatant was added 24 hr or more after the initiation of the cultures, the inhibition was not relieved. These results cannot be clearly interpreted, but it seems unlikely that lack of IL-2 is the main deficiency. Also, it is interesting to note that it has been reported that generation of suppressor macrophages *in vitro* can be prevented by addition to the cultures of mitogen-induced factors (Stout & Fisher, 1983b).

One previous set of data seems somewhat similar to the present MAb-induced inhibition, namely the report of macrophage hybridomas that inhibit T-cell responses at a level after IL-2 and IL-2 receptor interactions without any evidence for involvement of soluble factors (Ju & Dorf, 1985). As in the present case, the exact mechanism of the hybridoma inhibition is unknown.

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