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Augmentation of cell-mediated responses *in vitro* by a monoclonal anti-helper factor antibody

R. F. L. JAMES, G. H. SUNSHINE,* S. EDWARDS,† D. J. MAUDSLEY & M. FELDMANN *Imperial Cancer Research Fund, Tumour Immunology Unit, University College, London*

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Summary. Previous studies have shown that monoclonal antibody AF3.44.4 has specificity for a constant region determinant on mouse antigen-specific helper factors and that it also binds to cultured T cells with functional helper cell characteristics. The antibody synergizes with antigen to enhance *in vitro* antibody responses; here we demonstrate that it will also enhance cell-mediated responses *in vitro* such as in the generation of proliferating cells in mixed lymphocyte responses and in the generation of specific killer cells in cytotoxic T lymphocyte cultures. The mechanism of AF3.44.4-generated enhancement was investigated. Increased levels of the lymphokines IL-2 and BCDF were detected in supernatants of AF3.44.4-treated cultures but the antibody itself could not replace interleukin-2 (IL-2), and would not stimulate primed cells in the absence of antigen. This type of monoclonal antibody which augments immunological responses in an antigen-dependent fashion may provide a new class of immunostimulant and a new approach to augmenting the responses of weak immunogens.

* Present address: Department of Surgery, Tufts University Veterinary School, 136 Harrison Avenue, Boston MA 02111, U.S.A.

† Present address: Mount Sinai Hospital, 600 University Avenue, Suite 441, Toronto, Canada M56 1X5.

Correspondence: Dr R. F. L. James, Imperial Cancer Research Fund, Tumour Immunology Unit, University College London, Gower Street, London WC1E 6BT, U.K.

INTRODUCTION

One of the key questions in modern immunology concerns the nature of the receptor for antigen on T lymphocytes. We have previously approached this problem by studying the biochemical and functional characteristics of soluble material ('factors'), released by T cells, with antigen-binding properties (Feldmann & Kontiainen, 1981; Tada & Okumura, 1979). More recently we have raised monoclonal antibodies against these antigen-binding T-cell products with a view to obtaining further information about the antigen-specific receptor and its relationship to T-cell factors. We reasoned that antibodies directed against factor determinants shared by surface receptors should have profound effects on antigen-specific T-cell responses and we have shown that such antibodies will affect antigen-specific antibody responses (James *et al.*, 1983). Because of the overlap between antigen-specific syngeneic and allogeneic responses of T-cell clones (Haskins *et al.*, 1983), we further reasoned that anti-factor antibodies which affected the former type of responses should also influence the latter. This paper describes the effects of one such antibody, raised against soluble antigen-binding T-cell molecules, on cellular responses *in vitro* to alloantigens.

It has been shown previously that alloreactive T cells can be triggered by specialized accessory cells which express Ia antigens (Sunshine, Katz & Czitrom, 1982; Unanue, 1981). Stimulation by alloantigen

occurs in distinct phases (Larsson, 1982) leading to the activation of Lyt 1^+2^- helper cells which produce interleukin-2 (IL-2) (Shaw *et al.*, 1980) and induce Lyt 1^+2^+ or Lyt 1^-2^+ cytotoxic T-cell precursors to proliferate and subsequently differentiate into Lyt 1^-2^+ cytotoxic effector cells (Hardt *et al.*, 1981). Since these steps occur independently, we have been able to determine at which phase in the response this monoclonal antibody may act. We report here that AF3.44.4 significantly enhances the proliferative response of alloantigen-activated T cells. This is accompanied by enhanced production of IL-2, which in turn leads to high levels of cytotoxic T-cell induction in treated cultures. Enhanced levels of B-cell differentiation factor are also released and may account for the augmented antibody response previously reported (James *et al.*, 1983). As AF3.44.4 enhances helper effects in both antibody and cell-mediated responses *in vitro* it suggests that both systems share cells which bear the same antigenic determinant, and confirms that the helper cells for both responses are closely related (Bianchi *et al.*, 1981).

MATERIALS AND METHODS

Animals

Mice were obtained from the specific pathogen-free ICRF breeding colony at Burtonhole Lane, Mill Hill, London and maintained under pathogen-free conditions at University College London in Vickers Germ-Free Isolators (model SU-1).

Isolation of cells

Single cell suspensions of spleen, lymph node and thymus were prepared using nylon mesh after killing animals by cervical dislocation. To prepare cortisone-resistant thymocytes (CRT), mice aged 4–6 weeks were injected i.p. with 5 mg hydrocortisone acetate (Sigma, Poole, Dorset) 2 days before killing by exsanguination.

Monoclonal anti-helper factor antibody (AF3.44.4)

Spleen cells from a Wistar rat, hyperimmunized with Sepharose-KLH purified factor (Kontiainen & Feldmann, 1976) produced by DBA/2 mouse spleen cells *in vitro*, were fused with the HAT sensitive myeloma NS-I using the method of Köhler & Milstein (1975) as described previously (James *et al.*, 1983). Cells producing anti-HF antibody (AF3.44.4) were cloned by limiting dilution (Mishell & Shigi, 1980) and the (IgM κ) antibody purified by gel filtration and ion-exchange chromatography (Fahey & Terry, 1978) before use. Rat IgM, κ antibody YR43 (the gift of Dr I. McConnell) and rat anti-Lyt 1 (83.7.313) was used as a control.

Allogeneic responses (MLR)

All proliferation assays were set up in RPMI 1640 containing 5% heat-inactivated FCS, 10 mM HEPES, 2 mM L-glutamine and 5×10^{-5} M 2-mercaptoethanol for 4 days at 37° in an atmosphere of 5% CO_2. Flat-bottom microculture plates (Falcon, Oxnard, CA) were used with 4×10^5 lymph node cells or 3×10^4 CRT per well plus a suboptimal concentration (10^4 or 3×10^3 per well) of irradiated (2000 rads) spleen adherent cells (SAC) as stimulators (Czitrom, Katz & Sunshine, 1982). AF3.44.4 or rat IgM controls diluted in culture medium were added directly to microtitre wells and were present throughout the 4-day incubation. Each sample was set up in triplicate and 1 μCi ^{125}I-UdR was added to each well for the last 6 hr of incubation and the cells harvested on an automated cell harvester (Dynatech, Billinghurst, Sussex). Radioactive macromolecular DNA was assessed by gamma counting (Nuclear Enterprises, Reading, Berks). Bulk MLRs for phenotypic analysis were performed by incubating spleen cells from responder strain with irradiated (2000 rads) spleen cells from the stimulator strain for 7 days at 3×10^6 cells/ml in culture flasks.

Cytotoxic responses (CTL)

Cultures of responder lymph node cells were incubated in pentuplicate for 7 days in round bottom microtitre wells (Falcon, Cowley, Oxon) with irradiated SAC stimulator cells, as above. In some experiments an optimal concentration (1/100) of a standard IL-2 preparation derived from Con-A-stimulated spleen cells (provided by Dr T. Owens, University College, London) was also included. Replicate wells were then pooled and assayed for their ability to kill ^{51}Cr-labelled P815 cells or Con-A-induced blast cells depending on the relevant stimulator stain (Czitrom *et al.*, 1982). Results are expressed per 10^6 recovered cells or as lytic units per culture, where the lytic unit (LU) is defined as the reciprocal of the culture fraction which yields 50% specific lysis.

Assay for IL-2

Serial dilutions of test supernatants were assayed in

two systems with similar results: (a) the proliferation over a 24-hr period of 10^4 HT-2 cells, an IL-2-dependent mouse cell line (a gift of Dr R. H. Schwartz, NIH, Bethesda, MD, originally derived by Dr J. Watson) and (b) the enhancement of mitogen-dependent thymocyte proliferation (Gillis *et al.*, 1978). In this assay 5×10^5 thymocytes from 4–6-week-old mice were incubated for 3 days with phytohaemaglutinin (0·1 μg/ml). In both assays, proliferation was assessed by adding [^{125}I]UdR for the last 6 hr. A standard IL-2 preparation (see above) was always included for comparison. A linear increment of [^{125}I]UdR incorporation was seen over a dilution range of 1/20 to 1/4 of the supernatants.

Assay for B-cell differentiation factor 'BCDF'

This was performed as described by Schimpl & Wecker (1975). Briefly, 10^6 nude spleen cells from mice aged 6 weeks were cultured for 4 days in RPMI 1640 containing 5% FCS and 3×10^6 SRC in 96-well flat-bottom plastic plates (Linbro, Rickmansworth, Herts). Dilutions of rat Con-A supernatant or MLR supernatants generated with or without AF3.44.4 were assayed for their capacity to augment SRC responses. Plaque-forming cells were assayed by the Cunningham chamber method (Cunningham & Szenberg, 1968).

Flow cytometric analysis

Cortisone-resistant thymocytes were incubated with rat anti-Lyt 2 monoclonal antibody supernatant (provided by Dr B. Thomas, NIMR, London) 10 μg/10^6 cells followed by fluorescein-labelled sheep anti-rat Ig (Nordic Laboratories, Tilburg, Holland) and were then separated into Lyt 2^+ and Lyt 2^- populations using a FACS IV flow cytometer (Becton and Dickinson, Sunnyvale, CA). The proportion obtained corresponded closely to previously described values (Czitrom *et al.*, 1983) (e.g. approximately 35% of CRT were Lyt 2^+ compared with a value of approximately 75% Lyt 2^+ for untreated thymocytes). Lyt phenotypic analysis of AF3.44.4-positive cells was also performed on the FACS IV. Bulk MLR cultures (CBA anti-BALB/c) and unprimed CBA spleen cells were passed over nylon wool columns to collect the non-adherent cells (T cells) and then over Ficoll–Hypaque—yielding >95% viable cells. Cells for anti-Lyt killing were then incubated for 30 min on ice with anti-Lyt 1.1 or anti-Lyt 2.1 monoclonal antibodies (kindly provided by Prof. I. F. C. McKenzie, Melbourne, Australia) at 1/3000. After washing, the cells were then incubated for 30 min at 37° with Buxted Rabbit Complement (1/10) and were then washed before analysis on the FACS IV cell sorter.

RESULTS

The effect of AF3.44.4 on mixed lymphocyte responses

When added to cultures during the 4-day generation of an MLR, AF3.44.4 consistently enhanced allogeneic responses induced by allostimulator cells (Fig. 1a). Maximal enhancement (3–6-fold) was given by approximately 10 μg/ml antibody and the effect was lost at lower than 0·1 μg/ml antibody. Enhancement by AF3.44.4 was only seen in suboptimal conditions such as when the allostimulator cell population was used at 10^4 cells/well or lower (Table 1). Syngeneic responses were rarely enhanced by AF3.44.4 (and never more than 2-fold; Table 1, Fig. 1a) and primed T cells in secondary MLRs could not be stimulated with AF3.44.4 in the absence of antigen (data not shown). This confirms our previous report (James *et al.*, 1983) that the AF3.44.4 enhancement effect is dependent on the presence of antigen.

Enhancement of IL-2 production

Supernatants collected after the 4-day MLR cultures were tested for IL-2 production using an IL-2-dependent T-cell line HT-2. As demonstrated above, AF3.44.4 enhanced the proliferation of the allogeneic but not the syngeneic response (Table 2) and this enhancement was reflected in the levels of IL-2 produced. Thus no detectable IL-2 was found in the supernatants from the syngeneic response (with or without AF3.44.4 being present) but in the allogeneic response significant levels of IL-2 were seen. In the absence of AF3.44.4 (or in the presence of control antibody, YR43) the IL-2 level compares with a dilution of 1/2000 of the standard IL-2 preparation. In the presence of AF3.44.4 the IL-2 level was increased more than 2-fold (compares with a dilution of approximately 1/1000 of standard IL-2).

Enhancement of 'BDCF' release

Having shown that AF3.44.4 enhanced the release of IL-2, we also looked at its effect on lymphokines capable of augmenting B-cell responses (Table 3). Mixed lymphocyte responses (CBA anti-BALB/c) were set up as in Table 1 and the supernatants collected

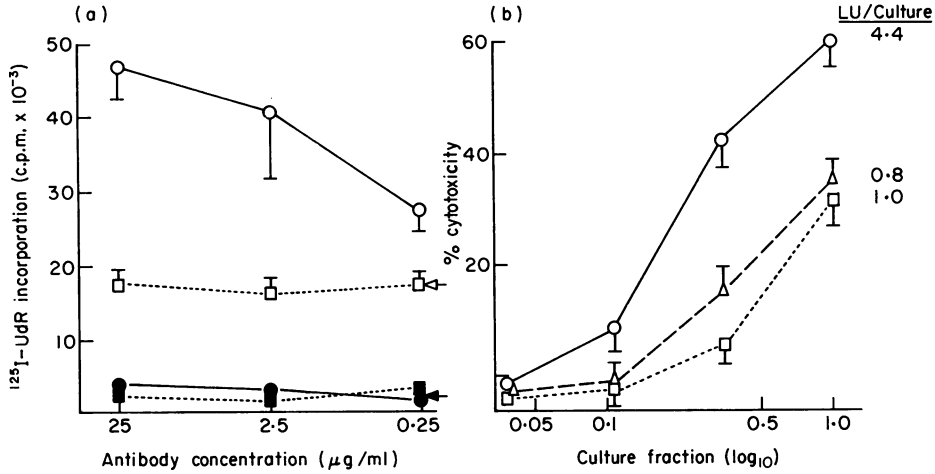

Figure 1. The effect of AF3.44.4 monoclonal antibody on *in vitro* cellular responses. (a) Mixed lymphocyte response. 4×10^5 responder lymph node cells (C57BL/10) were cultured with 10^4 allogeneic (B10.D2) or syngeneic (C57BL/10) irradiated SAC stimulator cells in triplicate 0·2 ml cultures. Purified AF3.44.4 (○, ●) or control antibody, YR43 (□, ■) were added to both allogeneic (○, □) and syngeneic (●, ■) combinations. In the absence of antibody, allogeneic (◂–) and syngeneic (–◂) responses are shown. Proliferation was measured by the uptake of ^{125}I-UdR into macromolecular DNA during the last 6 hr of culture. Results are plotted as mean ± SD. (b) Cytotoxic T-lymphocyte induction. Cultures of responder lymph node cells and irradiated SAC stimulator cells were set up in pentuplicate as above for 7 days and assayed for their ability to lyse ^{51}Cr-labelled P815 target cells. AF3.44.4 (○), 2·5 μg/ml; YR43 (□), 2·5 μg/ml; no antibody (△). Results are expressed as mean ± SD of a given culture fraction. Lytic units were calculated as described in methods. The addition of rat anti-Lyt 1 antibody (83.7.313) had no effect on either MLR or CTL induction and gave equivalent results to YR43.

on days 2 and 3. These were then assayed on nu/nu mouse spleen cell cultures. There was about 3-fold enhancement of 'BCDF' release in the presence of AF3.44.4 as compared with controls which could explain the augmented antibody responses reported previously (James *et al.*, 1983).

Enhancement of cytotoxic T lymphocyte induction

CTLs were generated over a 5-day culture period with or without AF3.44.4 and assayed on ^{51}Cr-labelled target cells (Fig. 1b). Control cultures gave a Lytic Index of approximately 1·0, while cells cultured in the

Table 1. The effect of AF3.44.4 on BALB/c anti-CBA mixed lymphocyte response

No. stimulator cells/well*	Syngeneic response†				Allogeneic response†			
	YR43‡	83.7.313‡	AF3.44.4‡	% Enhancement by AF3.44.4	YR43‡	83.7.313‡	AF3.44.4‡	% Enhancement by AF3.44.4
3×10^4	3096 ± 1226	2111 ± 579	2945 ± 709	−4·9	16,985 ± 772	15,884 ± 996	18,450 ± 8015	9·2
10^4	3037 ± 553	3987 ± 683	2676 ± 262	−11·9	10,148 ± 782	8636 ± 1210	25,873 ± 4134	154·9
3×10^3	3265 ± 708	2150 ± 727	3769 ± 959	15·4	2277 ± 226	4175 ± 856	14,564 ± 1674	539·6

AF3.44.4 enhances the proliferation of responding T cells in a mixed lymphocyte response (MLR) but only when suboptimal numbers of stimulator cells are used.

* Syngeneic (BALB/c) and allogeneic (CBA) spleen adherent cells were prepared as described previously (Sunshine *et al.*, 1982) and irradiated (2000 rads) before use.

† The proliferation of unprimed BALB/c lymph node cells after a 4-day culture period was measured by ^{125}I-UdR uptake and expressed as mean ± SD of triplicate cultures.

‡ Purified AF3.44.4, control antibody (YR43) or rat anti-Lyt 1 (83.7.313) were present at a concentration of 2·5 μg/ml throughout the 4-day culture period.

Table 2. Enhancement of IL-2 production by AF3.44.4

	MLR		IL-2 Assay	
Stimulus	Syngeneic	Allogeneic	Syngeneic	Allogeneic
10^4SAC	2910±433	20,507±529	209±143	3330±1654
+AF3.44.4	3495±849	57,079±1134	170±50	8106±995
+YR43	2336±145	20,810±471	174±19	3543±1181
+83.7.313	3161±274	22,857±812	283±23	3891±1251

In primary MLR cultures irradiated spleen adherent cells either syngeneic (CBA) or allogeneic (DBA/2) at 10^4/well were incubated with responding spleen cells (CBA) at 5×10^5/well for 4 days. Supernatant (50 μl) was removed from each well and added to a separate plate and incubated for 24 hr with HT-2 cells (5000 cells/well). Proliferation in both assays was measured by [^{125}I]UdR uptake and results are expressed as mean of triplicate cultures ± SD. A standard IL-2 preparation gave a response of 31 737 ± 1027 c.p.m. AF3.44.4, control antibody (YR43) and rat anti-Lyt 1 (83.7.313) were added at an optimal dose for AF3.44.4 (see Fig. 1a) of 2·5 μg/ml.

Table 3. Enchancement of BCDF release by AF3.44.4

		Response‡	
Antigen*	Supernatant†	AF3.44.4§	YR43§
−	—	0	
+	—	0	
+	25% Day 2	1226±255	552±115
+	5% Day 2	606±59	200±115
+	25% Day 3	956±131	360±189
+	5% Day 3	520±140	200±100

* Sheep red cells (3×10^6/well).

† Supernatants from CBA anti-BALB/c MLR taken on Day 2 or Day 3.

‡ Responses are expressed as the mean number of plaque-forming cells of triplicate cultures ± SD per 10^7 nude spleen cells.

§ The purified monoclonal antibodies AF3.44.4 and YR43 were present at 5 μg/ml only during the generation of MLR supernatants.

presence of AF3.44.4 produced more than 4 lytic units per culture and occasionally up to 6-fold enhancement of lysis was seen (Table 4). As the cellular pathway to CTL induction is dependent on the presence of IL-2 (Shaw *et al.*, 1980), the enhancement of cytolysis obtained with AF3.44.4 presumably reflects the increased IL-2 levels as seen in Table 2 although alternative possibilities exist (see Discussion).

By separating cortisone-resistant thymocytes on a FACS IV, we were able to investigate the differential

Table 4. The effect of AF3.44.4 on the generation of CTL from Lyt 2^+ and Lyt 2^- cell populations

Responder cells	IL-2*	AF3.44.4†	Cytolytic activity (LU/culture)
Unseparated	−	−	1·0
	−	+	6·5
	+	−	14·3
	+	+	15·6
Lyt 2^+	−	−	<0·2
	−	+	<0·2
	+	−	9·0
	+	+	18·0
Lyt 2^-	−	−	<0·2
	−	+	<0·2
	+	−	1·5
	+	+	1·4

CRT from CBA mice were stained with anti-Lyt 2 antibody and sorted on a FACS IV flow cytometer into Lyt 2^+ and Lyt 2^- subsets. Unseparated CRT were stained but not sorted. Responder CRT ($1{\cdot}5 \times 10^4$) were cultured in triplicate with 10^6 BALB/c spleen stimulator cells in the absence or presence of IL-2 or purified AF3.44.4. Cell recoveries and cytolytic activity on 2×10^4 Con-A-stimulated BALB/c spleen target cells were determined after 7 days in culture. Table shows representative of three separate experiments.

* An optimal concentration (1/100) of a standard batch of IL-2 was used throughout (see Materials and Methods).

† Purified AF3.44.4 was added at an optimal dose of 2·5 μg/ml.

effects of AF3.44.4 *in vitro* according to the Lyt 2 phenotype of the target cells (Table 4). In the presence of allogeneic stimulator cells, but in the absence of an exogenous source of IL-2, only the unseparated population generated cytolytic activity which was enhanced (6-fold) in the presence of AF3.44.4 (see Fig. 1b). Optimal cytolytic activity was induced by the addition of IL-2 to the unseparated CRT and AF3.44.4 had no additive effect (lines 3 and 4, Table 4). IL-2 also induced cytolytic activity in the Lyt 2^+ population (lines 7 and 8) which was enhanced by AF3.44.4. IL-2 alone or in combination with AF3.44.4 had little or no effect on the Lyt 2^- population (lines 11 and 12). Thus, AF3.44.4 cannot induce CTLs from Lyt 2^+ or Lyt 2^- populations alone but may act synergistically with IL-2 in the presence of CTL precursor cells (Lyt 2^+ population) to augment cytolytic activity.

Lyt phenotype of AF3.44.4 target cell

In previous experiments we have shown that AF3.44.4 binds (weakly) to T-cell hybrids and clones with helper cell phenotypes (James *et al.*, 1983). To determine the Lyt phenotype of the cells recognized by AF3.44.4 in mixed lymphocyte responses, allogeneic combinations of cells were set in 'bulk' culture for 7 days and the responding T cells purified over nylon wool columns. Some of these cells were then either treated with mouse monoclonal anti-Lyt 1.1 or anti-Lyt 2.1 antibodies plus complement and passed over Ficoll–Hypaque before being assayed for the presence of AF3.44.4, Lyt 1 and Lyt 2 positive cells (using monoclonal rat antibodies) on the FACS IV cell sorter (Fig. 2). Unprimed spleen cells have consistently been negative with AF3.44.4. However, *in vivo* (James *et al.*, 1983) and *in vitro* antigen-primed splenic T cells do show some positivity and approximately 20% of these cells now bind AF3.44.4 (Fig. 2). After treatment with anti-Lyt 1.1 and complement all AF3.44.4-binding cells were removed. Treatment with anti-Lyt 2.1 removed a proportion of positive cells, but not all. Thus, phenotypically, AF3.44.4-positive cells may be Lyt 1^+2^- or Lyt 1^+2^+.

DISCUSSION

In a previous publication (James *et al.*, 1983) we have shown by affinity column elution that a monoclonal antibody raised against antigen-specific helper factor

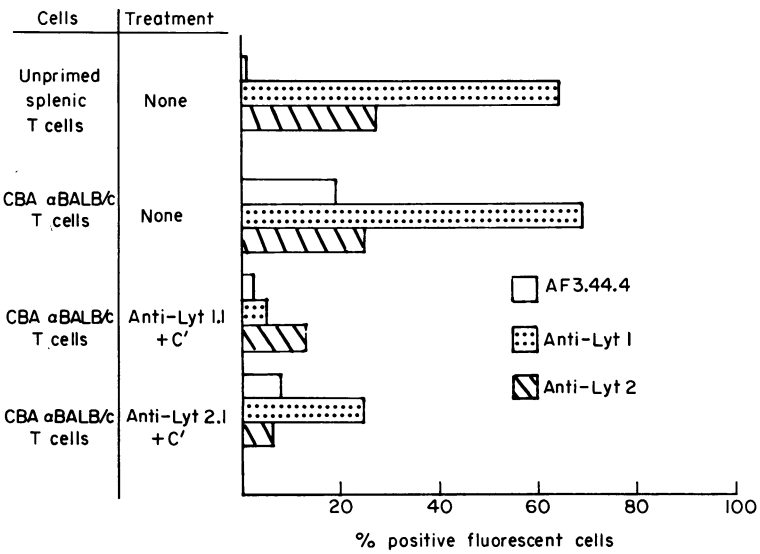

Figure 2. Lyt phenotype of AF3.44.4-positive cells. Nylon-wool passed CBA spleen cells were either left untreated or incubated with monoclonal anti-Lyt 1.1 or anti-Lyt 2.1 followed by rabbit complement. The cells were then incubated with AF3.44.4 or monoclonal rat anti-Lyt 1 or anti-Lyt 2 antibody supernatants followed by FITC-conjugated sheep anti-rat Ig before being washed and analysed on a FACS IV cell sorter. Percentage positive cells (−background staining with irrelevant rat IgM monoclonal antibody YR43) is shown for AF3.44.4, Lyt 1 and Lyt 2. Figure shows representative of three separate experiments.

defines a constant region on such factors and, in appropriate circumstances, acts on helper T cells to augment the production of antibody by B cells. Here we report that this same monoclonal antibody also enhances *in vitro* allogeneic responses: both allospecific proliferation and the induction of cytotoxic T cells were specifically enhanced in all allogeneic combinations tested. The augmenting effect bears two distinctive characteristics (a) it occurs only in the presence of antigen (i.e. alloantigen; Table 1, Fig 1a) and (b) it is only apparent in suboptimal responses such as when low concentrations of stimulator alloantigen are present (Table 1).

The mechanism of these effects was analysed. We found that AF3.44.4 augmented the release of lymphokines by antigen-activated T cells [e.g. IL-2 (Table 2) and B cell-differentiating factor (BCDF) (Table 3)] and since IL-2 is required for the induction of T-cell cytolysis and T-cell proliferation (Shaw *et al.*, 1980; Hardt *et al.*, 1981) it is probable that augmented IL-2 release is the mechanism by which AF3.44.4 enhances cell-mediated responses.

The cellular site of action of AF3.44.4 was also explored. Though AF3.44.4-binding cells were undetectable among normal splenic T cells significant numbers were found after alloantigen stimulation: 20% of CBA cells recovered after priming for 7 days *in vitro* with BALB/c spleen cells now bound AF3.44.4 (Fig. 2). The predominant phenotype of such AF3.44.4 positive T cells was Lyt 1^+2^- which generally defines the helper cell population in alloantigen responses (Glasebrook *et al.*, 1983) but a minor population was Lyt 1^+2^+. Both of these T-cell subsets have been described as capable of producing IL-2 in response to alloantigen (Glasebrook *et al.*, 1983; Widmer & Bach, 1981) and it is possible that AF3.44.4 may augment IL-2 production by either or both populations of T cells. It is noteworthy, however, that AF3.44.4 can synergize with IL-2 to enhance the differentiation of purified Lyt 2^+ cells to CTL in the absence of Lyt 1^+2^- cells (Table 4). Since Table 4 also illustrates that AF3.44.4 does not mimic the effects of IL-2 on the Lyt 2^+ population, we suggest that AF3.44.4 may also act in the presence of stimulator alloantigen on a small population of Lyt 1^+2^+ cells to augment the effect of exogenous IL-2.

Our previous studies with antisera and monoclonal antibodies raised against antigen-specific factors have indicated that soluble factors released by T cells bear 'constant region' or 'isotypic' determinants which are shared by molecules on the surface of T cells (Culbert *et al.*, 1982). The results presented here, and previously (James *et al.*, 1983) suggest that AF3.44.4 recognizing antigen-specific helper factor and helper T cells, may react with a constant region of helper T-cell receptors. Some studies have shown that antibodies recognizing T cell-receptor idiotype structures will induce IL-2 release from T-cell clones (Meuer *et al.*, 1983) or T-cell hybrids (Haskins *et al.*, 1983) but only when coupled to Sepharose beads. However, there is now much evidence to show that soluble antibodies with both 'idiotypic' and 'constant region' specificities for T-cell surface structures (Samelson & Schwartz, 1984; Kaye *et al.*, 1983; Gunter, Malek & Shevach, 1984; Meuer *et al.*, 1984) can stimulate T-cell clones and T-cell hybrids. Whilst the effect of AF3.44.4 is very similar to these antibodies, the requirement for the simultaneous presence of antigen needed for the AF3.44.4 enhancing effect (Tables 1 and 2, Fig. 1) is not found in other systems.

The molecular mechanism of AF3.44.4 enhancement is still under study but we think that it may crosslink receptors and enhance the effect of antigen: consistent with this are observations that monoclonal anti-receptor antibodies which are inhibitory in soluble form are stimulatory after coupling to Sepharose beads (Haskins *et al.*, 1983; Meuer *et al.*, 1983). Regardless of the actual mechanisms involved, the dependence of the enhancing effects on antigen gives AF3.44.4-specific immunomodulating properties.

In summary, AF3.44.4 is a monoclonal antibody which binds to helper T cells and, in an antigen-dependent fashion, augments the release of the T-cell effector molecules IL-2 and BCDF. It thus provides a new approach to enhancing responses to weak immunogens.

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