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Induction of interleukin 2 receptiveness and proliferation in resting peripheral T cells by monoclonal anti-CD3 (T3) antibodies does not require the presence of macrophages

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SUMMARY

In this study, we sought to elucidate the sequence of events by which mitogenic monoclonal anti-CD3 antibodies (anti-CD3-MoAb) initiate T cell activation. In cultures of monocyte-depleted resting T cells, two anti-CD3-MoAb, OKT3 and anti-Leu 4, induced a state of interleukin 2 (IL-2) receptiveness which culminated in T lymphocyte proliferation when recombinant IL-2 was provided. Evidence that Fc-receptor mediation by monocytes did not contribute to this mitogenesis was supported by studies showing that polyclonal $F(ab')_2$ anti-mouse IgG Fc antibody did not alter the magnitude of the IL-2 driven T cell proliferative response, and by the use of T cells from donors whose monocytes were unable to assist in the induction of anti-Leu 4 (IgG1 subclass) initiated proliferation. Anti-CD3-MoAb, in the absence of IL-2, induced IL-2 receptor expression on purified T cells, and anti-IL 2 receptor antibodies inhibited T cell proliferation in the presence of this growth factor. Furthermore, following modulation of the CD3 molecular complex in the presence of monocytes, depletion of accessory cells rendered the modulated T cells mitogenically dependent on exogenous IL-2. IL-2 itself did not suffice to promote T cell proliferation in the absence of anti-CD3-MoAb. These results indicate that the binding of monoclonal antibody to CD3 is capable of initiating, in an accessory cell-independent manner, premitotic alterations in T cells which can culminate in proliferation when exogenous IL-2 is provided.

Keywords T cells CD3 IL-2-receptor IL-2

INTRODUCTION

The T cell antigen receptor is a complex composed of a clonotypic 90 kD heterodimer (Ti) and a 20–25 kD complex termed CD3 (T3) (Meuer *et al.*, 1983a, b). Numerous studies have confirmed the view that CD3 is of major importance in T cell responses, probably acting as a transducer of antigenic signals from the cell surface to the cytosol. Monoclonal antibodies (MoAb) directed at CD3 (anti-CD3-MoAb), e.g. OKT3 and anti-Leu 4, have been shown to inhibit antigen-specific proliferation, cytotoxic effector function, and lectin-induced mitogenic T cell proliferation (Meuer *et al.*, 1983a; Landgren *et al.*, 1982; Tsoukas *et al.*, 1982; van Wauwe, Goossens & van Nyen, 1984). Moreover, under appropriate conditions, these anti-CD3 MoAb themselves induce T cell mitogenesis (van Wauwe, De Mey & Goossens, 1980; Chang *et al.*, 1981).

The mitogenicity of anti-CD3 MoAb has been regarded to be dependent upon multimeric cross-

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linkage of the T cell antigen receptor complex—a function which can be provided by Fc receptor bearing accessory cells, i.e. monocytes (MΦ) (Chang *et al.*, 1982) or, in the case of certain T cell clones, by the coupling of MoAb to Sepharose beads (Meuer *et al.*, 1983c). The critical role of multimeric cross linkage has been further supported by studies employing peripheral blood mononuclear cells (PBMC) which have shown that $F(ab')_2$ and Fab fragments of anti-CD3—MoAb do not effectively induce T lymphocyte proliferation (Chang *et al.*, 1982). Whether the binding of anti-CD3 antibody to resting T cells in the absence of MΦ can elicit activation has not been fully clarified. In the present study, we have analysed this subject.

MATERIALS AND METHODS

Cell populations. PBMC were isolated from buffy coats or heparinized peripheral blood of healthy donors by gradient centrifugation over Ficoll-Paque (Pharmacia, Uppsala, Sweden). The interface cells were collected, washed three times and suspended in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum (FCS), 2 mM L-glutamine, 10 mM HEPES buffer, 0·1% sodium bicarbonate, 100 U/ml penicillin and 100 μg/ml streptomycin (all from GIBCO, Frankfurt, FRG). Separation of PBMC into monocyte- and lymphocyte enriched cell populations was performed by adherence on plastic Petri dishes (Costar, Badhoevedorp, The Netherlands) for 1 h at 37°C in a humidified atmosphere of 5% CO_2 in air. For T cell enrichment, non-adherent cells were washed, resuspended in RPMI 1640, mixed with a sheep red blood cell (SRBC) suspension and centrifuged over Ficoll/Paque as previously described (Smolen *et al.* 1981). The SRBC contained in the pellet were lysed with ammonium chloride buffer and washed twice with medium.

This T-enriched cell population was then further depleted of non-T cells by two cycles of cytolysis using a cocktail of antibodies directed against antigen specificities expressed on non-T cells (see below) and a 1:8 dilution of rabbit complement (C′) (Cederlane Laboratories Ltd, Hornby, Ontario, Canada). The T cells were finally washed and resuspended to appropriate concentrations in culture medium (CM) (medium as described above, but containing 5% FCS). The highly purified T cell populations contained < 1% contaminating MΦ according to flow cytometry studies (see below).

For experiments involving the addition of MΦ, plastic-adherent cells were incubated with 50 μg/ml mitomycin C (Sigma, St Louis, MΦ) in CM for 30 min at 37°C. The cells were then harvested using a cell scraper (Costar), washed extensively, and resuspended to appropriate concentrations in CM.

Antibodies. MoAb of the Leu series and a fluoresceine-isothiocyanate (FITC) conjugated IgG1 monoclonal anti-IL-2-receptor antibody were purchased from Becton Dickinson (Mountain View, CA), OK-series MoAb from Ortho Pharmaceuticals (Raritan, NJ). Anti-Tac, an IgG2a MoAb against the IL-2 receptor (IL-2R) (Uchiyama, Broder & Waldmann, 1981; Leonard *et al.*, 1982), was a generous gift of Dr T. A. Waldmann, Bethesda, MD. A polyclonal $F(ab')_2$ fragment of goat anti-mouse IgG-Fc was obtained from Cappel Laboratories (Cochranville, PA). An FITC $F(ab')_2$ fragment of goat anti-mouse Ig (FITC-GAM) (GRUB, Vienna, Austria) was used in indirect immunofluorescent studies.

For T cell purification, OKM1, OKB7, a monoclonal IgM anti-HLA-DR antibody (Seralab, Accurate Chemical and Scientific Co., Westbury, NY) and a polyclonal rabbit anti-mouse immunoglobulin preparation (Dakopatts, Glostrup, Denmark) were used. T cells were analysed by flow cytometry after staining with FITC-anti-Leu M3 and FITC-anti-HLA-DR.

Where appropriate, antibodies were dialysed against two changes of RPMI 1640 medium for 24 h at 4°C to remove sodium azide and were sterile filtered.

Cytofluorographic analysis. The expression of cell surface antigens were determined by flow cytometry by means of direct or indirect immunofluorescence as previously described (Smolen, Sharrow & Steinberg, 1981). After staining, washing and resuspension, cells were immediately analysed on a FACS 440 (Becton Dickinson) to determine the fluorescence intensity and forward and 90° light scatters.

Proliferation assays. Triplicate or quadruplicate cultures containing 10^5 T cells with or without the addition of 10^5 autologous, mitomycin C-treated MΦ were established in flat bottom microtitre plates (Costar) in CM. In some experiments, 10^5 unfractionated PBMC were used. The mitogenic MoAb anti-Leu4 or OKT3 were added to the cultures in concentrations of 2·5–200 ng/ml. The lectin mitogen concanavalin A (Con A) (Pharmacia) was added to certain cultures in a dose of 2 μg/ml (optimal mitogenic concentration according to preliminary experiments).

Recombinant IL-2, purified to homogeneity (specific activity 2×10^6 U/mg protein; AMGen Biologicals, Thousand Oaks, CA) was used in concentrations of 20–200 U/ml. In some experiments, $F(ab')_2$ anti-mouse IgG-Fc was added to cultures at a 1:400 or 1:800 dilution.

For preculture experiments, T cells (4×10^6/ml) were preincubated with IL-2 (200 U/ml) or anti-CD3 MoAb (200 ng or 1 μg/ml) for 18 h at 37°C. The cells were then extensively washed and recultured in microtitre plates with or without the addition of MoAb and/or IL-2. In another series of experiments, we precultured 4×10^6 PBMC/ml with OKT3 or anti-Leu4 (200 ng/ml) for 18–40 h and then performed cytolysis of non-T cells as described above to yield a population of purified CD3-modulated T lymphocytes (Tm). Tm were then employed in a second culture system with or without the addition of MoAb and/or IL-2.

All cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air for time periods stated in the Results section. Six hours before termination, cultures were pulsed with 1 μCi of tritiated thymidine (^{3}HTdR, Amersham, Buckinghamshire, UK). Cultures were harvested onto glass-fibre filter papers with an automated harvester, and thymidine incorporation was measured in a liquid scintillation counter (Beckman, Chicago, IL). Results were expressed as Δ d/min (disintegrations per minute), i.e. mean d/min of experimental cultures minus mean d/min of cultures in medium alone. Standard errors of the mean did not exceed 10% and have been omitted for clarity.

RESULTS

Anti-CD3-MoAb induce the proliferation of purified, peripheral T cells in the presence of IL-2. In initial experiments we sought to address the question if resting T cells could mount a mitogenic response to OKT3 or anti-Leu4 in the absence of MΦ. For this purpose, highly purified T cells which contained < 1% MΦ according to flow cytometry analyses were incubated with either of these MoAb or Con A in the presence or absence of exogenous, recombinant IL-2. As shown in

Table 1. Purified T cells proliferate to monoclonal antibodies to the CD3 complex in the presence of IL-2

Exp.	IL-2*	Con A	OKT 3	Anti-Leu4	Medium
1	–	0†	300	400	—
	+	5,500	11,800	11,600	400
2	–	200	0	0	—
	+	3,900	8,500	10,800	600
3	–	0	0	0	–
	+	17,300	5,200	10,500	400
4	–	100	ND	200	—
	+	8,200	—	16,500	200
5	–	100	0	0	—
	+	14,500	1,900	5,200	300

* Final concentrations in cultures of 10^5 T cells: IL-2: 200 U/ml, Con A: 2 μg/ml; OKT3: 200 ng/ml (Exp 1–2) or 50 ng/ml (Exp 3–5).

† Mean Δd/min ^{3}H TdR (to nearest hundred) of triplicate cultures after a 96 h incubation period.

ND, Not done.

Table 1, purified T cells failed to respond to MoAb or Con A alone, but displayed significant proliferative activity if exogenous IL-2 was added. The magnitude of the response was dependent on both the doses of MoAb and the amount of IL-2; optimal responses were obtained at IL-2 concentrations of 100–200 U/ml and at 50–200 ng/ml of the MoAb (data not shown). These data suggested that resting T cells did not require MΦ to proliferate in the presence of anti-CD3 MoAb, if exogenous IL-2 was provided.

MΦ contamination does not account for the response of highly purified T cells to OKT3 or anti-Leu4 in the presence of IL-2. Although the purified T cell populations employed did not contain MΦ according to flow cytometry analyses and as evident by their incapacity to mount substantial proliferative responses to Con A, it was possible that the proliferation observed in the presence of IL-2 was dependent upon a minute MΦ contamination of the purified T cell population. In order to exclude this possibility, we took advantage of two previous observations: (1) the function of MΦ in the context of anti-T3 induced T cell activation has been shown to be inhibitable by blocking Fc binding (Looney & Abraham, 1984); and (2) there exists a large number of individuals unresponsive to the IgG1 MoAb anti-Leu4 (but not to the IgG2a MoAb OKT3) (Van Wauwe & Goossens, 1983); this unresponsiveness has been related to a deficiency of MΦ receptors for murine IgG1 (Tax *et al.*, 1984).

In a series of experiments, we analysed the effects of the $F(ab')_2$ fragment of a polyclonal goat anti-mouse IgG-Fc antibody preparation. As can be seen in Fig. 1, this antibody preparation was able to essentially abrogate the proliferative response of PBMC to mitogenic MoAb (but not to the lectin mitogen Con A). Thus, if potential minimal MΦ contamination of highly purified T cells were responsible for triggering signals in the experiments employing MoAb plus IL-2, significant inhibition of proliferation by the $F(ab')_2$ anti-Fc antibody would be expected. However, the addition of the second antibody to cultures of pure T cells, mitogenic MoAb and IL-2 did not significantly alter the magnitude of the response (Fig. 1).

The second series of experiments involved the use of T cells and MΦ from anti-Leu4 non-

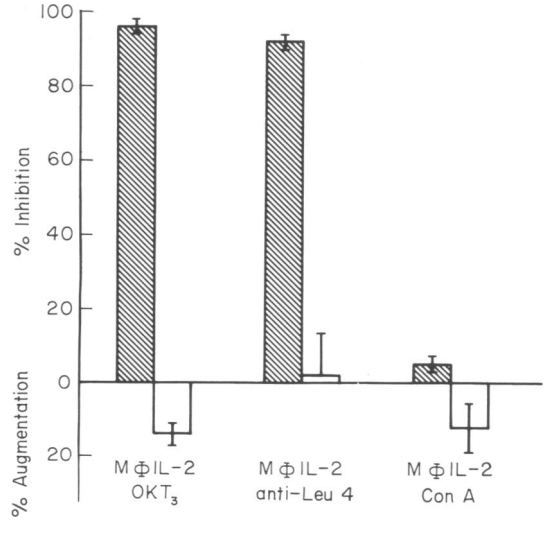

Fig. 1. $F(ab')_2$ anti-mouse Fc-IgG does not alter the ability of IL-2 to promote mitogenic T cell responses. An $F(ab')_2$ fragment of goat anti-mouse Fc-IgG was added to 10^5 T cells and optimal concentrations of mitogenic MoAb or Con A in the presence of either 10^5 autologous, mitomycin C-treated MΦ (shaded bars) or 200 U/ml recombinant IL-2 (open bars); % inhibition was calculated as follows:

$$\left(1-\frac{\text{mean } \Delta \text{ d/min } {}^3\text{HTdR for triplicate culture containing } F(ab')_2 \text{ antibody}}{\text{mean } \Delta \text{ d/min } {}^3\text{HTdR for triplicate cultures without } F(ab')_2 \text{ antibody}}\right)\times 100$$

Augmentation = 'negative' inhibition. Results are means of five experiments ±s.e.m.

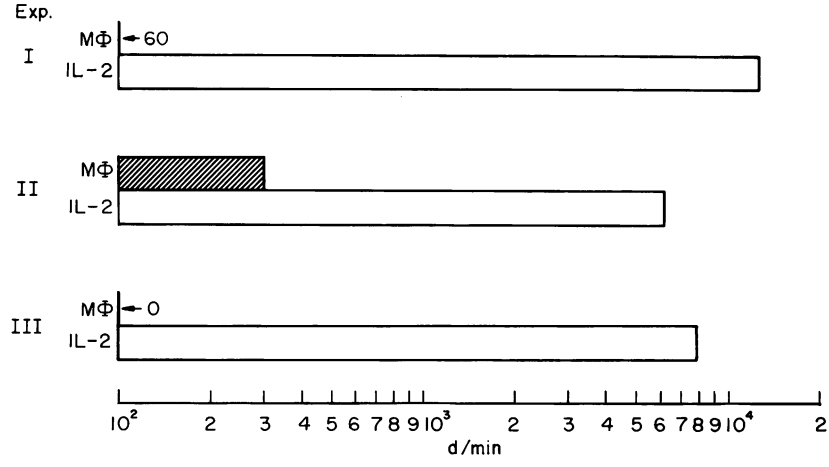

Fig. 2. T cells from anti-Leu4 non responders proliferate in the presence of monoclonal anti-Leu4 when provided with exogenous IL-2. T cells from anti-Leu4 non-responders proliferate in the presence of anti-Leu4 when provided with exogenous IL-2. 10^5 T cells were supplemented with either 10^5 mitomycin-C treated, autologous MΦ or 200 U/ml recombinant IL-2 in the presence of 200 ng/ml anti-Leu4 for 96 h. Results represent Δ d/min ^{3}HdR of triplicate cultures. Three representative experiments performed under identical conditions are depicted.

responder individuals. As shown in Fig. 2, highly purified T cells from such individuals proliferated to anti-Leu4 when exogenous IL-2 was provided; thus, even if their purified T cells were minimally contaminated with MΦ, these would not have been able to induce the T cell proliferation we observed.

Taken together, these data virtually excluded the participation of any contaminating MΦ in the response of purified T cells to anti-CD3 MoAb in the presence of IL-2.

Anti-CD3 MoAb, but not IL-2 alone, renders T cells receptive to subsequent action of IL-2. The results presented suggested that although the binding of MoAb to the T3 antigen in the absence of multimeric cross linkage did not suffice to induce T cell proliferation, it did elicit a state of IL-2-responsiveness in these cells. We assumed therefore that the binding of the MoAb induced IL-2R expression on the resting T cells.

The fact that no substantial thymidine incorporation by T cells occurred in cultures containing only IL-2 spoke against a major role of IL-2 in the initial induction of IL-2R in MoAb-free cultures. However, the simultaneous presence of IL-2 and anti-CD3-MoAb in the culture did not allow us to exclude the possibility that both of these factors were contributing to the initiation of T cell IL-2 receptiveness.

In order to address this point, we preincubated highly purified T cells for 18 h at 37°C in CM alone or CM containing either IL-2 or anti-Leu4. Following preincubation, the cells were washed and cultured for 72 h with or without the addition of IL-2 and/or anti-CD3-MoAb. As shown in Fig. 3, preincubation with medium alone or with medium containing IL-2 did not induce subsequent IL-2 or anti-T3 responsiveness. In contrast, when cells were first incubated with the MoAb, they exhibited a proliferative response only when exogenous IL-2 was present. Thus, preincubation with anti-CD3-MoAb rendered resting T cells receptive to IL-2, but not vice versa.

However, when anti-CD3-MoAb-preincubated T cells were analysed by flow cytometry, we observed that all OKT3 preincubated cells and approximately 65% of anti-Leu4 preincubated cells, still bore surface-bound MoAb. Thus, it was possible that IL-2 was only capable of exerting its effects in the continuous presence of membrane-bound MoAb. In order to effect modulation and, therefore, to eliminate membrane bound antibody, we took advantage of the ability of MΦ to assist modulation (i.e. elimination from the surface) of the CD3 complex in the presence of anti-CD3-MoAb (Rinnooy Kan *et al.*, 1984). When PBMC were preincubated for 18 h with anti-CD3-MoAb,

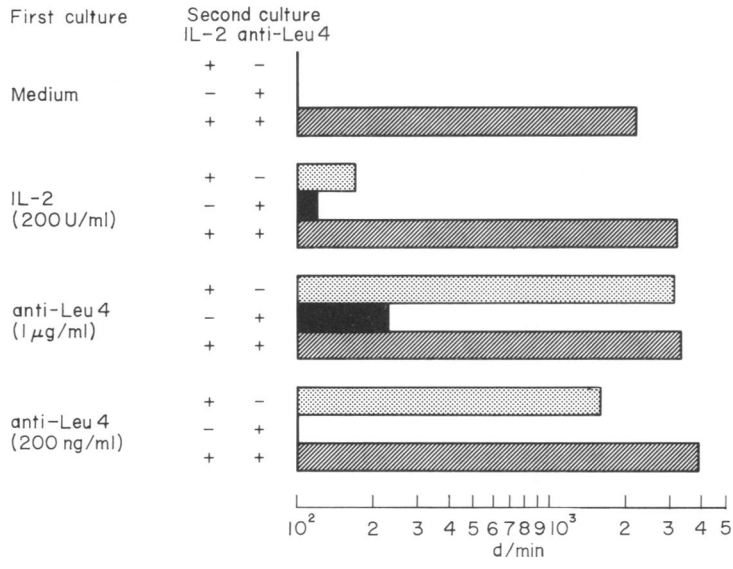

Fig. 3 Preincubation with monoclonal anti-Leu4 antibody renders T cells IL-2 responsive. T cells were preincubated for 18 h at 37°C in the presence of either CM alone or CM supplemented with IL-2 or anti-Leu4 in the doses indicated. Following the preincubation period, cells were extensively washed and employed in a second culture system containing the same supplements (anti-Leu4, 200 ng/ml) for 72 h at 37°C. Results are expressed as mean Δ d/min ^{3}HTdR of triplicate cultures.

Table 2. Proliferation of purified anti-T3 modulated T cells requires exogenous IL-2

Addition to Tm* in second culture			Preculture conditions*		
IL-2†	OKT3	anti-Leu4	OKT3	OKT3	Anti-Leu 4
−	−	−	742‡	157	127
−	+	−	906	361	106
−	−	+	739	520	561
+	−	−	10,839	9,702	8,887
+	+	−	7,768	34,828	30,234
+	−	+	5,652	26,306	34,848

* Tm were isolated by eliminating non-T cells after preincubation of PBMC for 18 h with 200 ng/ml of the MoAb indicated. Three representative experiments are shown.

† IL-2, 100 U/ml; OKT3, 200 ng/ml; anti-Leu4, 200 ng/ml.

‡ Mean d/min^3 HTdR for triplicate cultures of 10^5 Tm cells harvested 72 h after initiation of the second culture.

flow cytometric studies revealed complete modulation (data not shown). Following accessory cell depletion. the modulated T cells (Tm) were employed in second cultures to which either CM alone or anti-CD3 MoAb and/or IL-2 were added. When harvested 24–72 h after initiation of the second cultures, these Tm did not proliferate unless an exogenous source of IL-2 was provided (Table 2). This mitotic dependency upon exogenous IL-2 was not overcome by the addition of anti-CD3-MoAb.

Table 3. Blocking of T cell proliferative responses by monoclonal antibodies to the IL-2 receptor

	Con A + IL-2*	Anti-Leu4 + IL-2*
Medium	15,001†	24,760
anti-IL-2R 12·5 ng/ml	1,488 (90%)‡	2,039 (92%)
anti-IL-2R 6·2 ng/ml	5,803 (61%)	6,132 (75%)
anti-TAC 1:4000	0 (100%)	0 (100%)
anti-TAC 1:8000	1,979 (87%)	5,380 (78%)

* IL-2, 200 U/ml; Con A, 2 μg/ml; anti-Leu4, 200 ng/ml.

† Mean $\Delta d/min^3TdR$ of triplicate cultures of 10^5 T cells after a 96 h culture period.

‡ Percent inhibition.

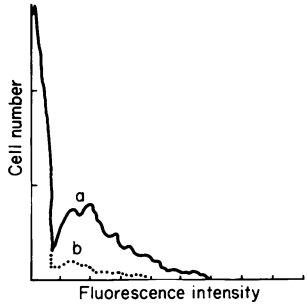

Fig. 4. IL-2R expression of anti-T3 preincubated T lymphocytes. T cells were incubated for 18 h in CM containing 200 ng/ml OKT3 MoAb either at 37°C (—, a) or at 4°C in the presence of 0·02% NaN_3 (·····, b), stained with FITC labelled anti-IL-2R and analysed by flow cytometry. T cells incubated at 37°C in CM in the absence of OKT3 showed a profile similar to 'b' (not shown).

The IL-2 receptor is involved in the proliferative response of T cells to anti-CD3-MoAb in the presence of IL-2. Since IL-2-mediated T cell proliferation has been shown to depend upon the expression of membrane IL-2R (Leonard *et al.*, 1982; Robb, Munck & Smith, 1981), antibodies to the IL-2R should block the T proliferative response induced by anti-T3 MoAb plus IL-2. Both a commercially available anti-IL-2R-MoAb as well as anti-Tac inhibited, in a dose-dependent fashion, the proliferation of T cells to anti-CD3 mitogenic stimuli or Con A in the presence of IL-2 (Table 3). When the purified T cells which had been preincubated with anti-T3-MoAb for 18 h at 37°C were stained with FITC-anti-IL-2R MoAb, IL-2 expression was detected on up to 10% of cells as compared to $< 2\%$ of cells incubated at 4°C in the presence of 0·2% sodium azide under identical conditions (Fig. 4).

DISCUSSION

In this study we describe the ability of anti-CD3-MoAb to render highly purified peripheral T cells sensitive to IL-2 and to induce proliferation in the absence of MΦ. Since mitogenicity of the MoAb OKT3 and anti-Leu4 has been regarded to be strictly dependent upon MΦ which mediate their effect primarily via their Fc receptors (Chang *et al.*, 1982; Looney & Abraham, 1984; Tax *et al.*, 1984), we had to ensure that the results observed were not due to minute MΦ contamination. Such

evidence was obtained by demonstrating (i) the lack of contaminating MΦ according to flow cytometry; (ii) that T cells did not respond to the MΦ dependent mitogen Con A; (iii) that in the presence (but not absence) of MΦ anti-CD3 induced proliferation could be inhibited by blocking Fc-mediated effects; and (iv) that T cells from anti-Leu4 non-responders proliferated well to anti-Leu4 in the presence of IL-2. Moreover, preliminary data of Holter *et al.* (1985) revealed analogous effects of an IgM-anti-CD3-MoAb in the presence of IL-2. Our data extend previous observations on the ability of anti-CD3-MoAb to render PBMC or MΦ supplemented T cells responsive to IL-2 (Welte *et al.*, 1984; Tsoukas *et al.*, 1985) and provide further evidence that T cell activation via the T3/Ti complex can occur in the absence of accessory cells if IL-2 is provided. The IL-2 receptiveness was mediated by the emergence of IL-2R as indicated by (i) flow cytometry and (ii) blocking studies with anti-IL-2R-MoAb. IL-2 may promote anti-CD3 induced T cell growth in a manner similar to phorbol ester (Holter *et al.*, 1985; Hara & Fu, 1985). In this regard, a recent study has shown that IL-2 and phorbol ester induce protein kinase C transposition in an analogous manner (Farrar & Anderson, 1985).

Further experiments in which T cells were purified after complete modulation of the CD3-antigen (and thus also elimination of the surface bound MoAb) revealed that the addition of IL-2 alone, but not MoAb alone, sufficed to drive such preactivated T cells into proliferation. Under these conditions, simultaneous signalling by both the antibody and the growth factor apparently was not necessary, nor had the preactivation procedures induced proliferation autonomy. The data indicate that the modulated cells quickly lose their ability to produce IL-2 following the elimination of stimulatory signals, but maintain their receptiveness to IL-2. Thus, T cells pre-incubated with MoAb to the CD3-antigen achieve IL-2 receptiveness under modulating and non-modulating conditions.

With regard to a potential role of the MΦ-product IL-1 in the context of the proliferative responses observed, IL-1 was (a) not detected in supernatants of highly purified T cells cultured with anti-CD3-MoAb (data not shown), and (b) did not induce significant T cell activation in the presence of soluble anti-CD3-MoAb both in our hands (data not shown) and in another study (Williams *et al.*, 1985); in some investigations (Williams *et al.*, 1985; Meuer & Meyer zum Büschenfelde 1986) IL-1 did, however, induce T cell activation if Sepharose coupled anti-CD3-MoAb was used, a finding compatible with a modifying role of IL-1 in the event of crosslinkage of the CD3-antigen.

It has been recently shown that following T cell activation, the presence of IL-2 was necessary for optimal surface expression of IL-2R (Welte *et al.*, 1984; Smith & Cantrell, 1985; Rem & Yeh, 1984). This phenomenon might have played an additional role in our experiments as also noted by Meuer & Meyer zum Büschenfelde (1986) using anti-CD3-coupled beads. Nevertheless, our data indicate that IL-2, in the absence of MoAb, could not induce a state of preactivation. Further, in contrast to supernatants of PBMC, no IL-2 was detectable in supernatants of purified T cells cultured with anti-CD3-MoAb (data not shown). In this regard, purified peripheral T cells may be similar to the T3-positive human T cell line Jurkat (Weiss, Wiskocil & Stobo, 1984): the mere binding of OKT3 to these cells was not sufficient to induce IL-2 synthesis nor appreciable expression of IL-2 RNA.

In conclusion, our data provide evidence that interaction of anti-CD3 MoAb with the T cell membrane induces the emergence of IL-2 receptors and receptiveness to IL-2. Our observations imply that neither multimeric crosslinking of the T cell antigen receptor nor the presence of MΦ (or their products) are critical in the initial phase of T cell activation via the T3/Ti complex. Moreover, provision of exogenous IL-2 bypasses the need for MΦ in the proliferative response. Since the CD3 moiety is part of the T cells' antigen-receptor complex and since the action of anti-CD3-MoAb is indistinguishable from that of MoAb to the antigen receptor Ti and also similar to that of nominal antigen (Meuer *et al.*, 1983a), it is conceivable that these findings also apply to stimuli other than MoAb and thus may have important physiological implications.

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