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Signal delivery by physical interaction and soluble factors from accessory cells in the induction of receptor-mediated T-cell proliferation. Synergistic effect of BSF-2/IL-6 and IL-1

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SUMMARY

Our recent study revealed that soluble factors derived from accessory cells (AC; monocytes) and physical interaction with T cells of the accessory cells are both required for the induction of the proliferation of human peripheral blood T cells by anti-CD3 antibody coupled on latex beads. The accessory cell-derived soluble factor could be replaced by IL-1 and IL-6, and the role of live macrophages for physical interaction with T cells was found to be replaceable with paraformaldehyde(PFA)-fixed macrophages, provided the macrophages were pretreated with interferon-gamma (IFN-γ) before fixation. Quantitative analysis in the present study revealed that IL-1 and IL-6 act synergistically to induce T-cell proliferation in the above system but either one of the factors alone reveals only a marginal or weak activity. Furthermore, it was shown that the potentiating activity of the culture supernatants of monocytes was substantially inhibited by anti-IL-6 antibody. Taken together with our previous results that anti-IL-1 serum strongly inhibited the potentiating activity of the culture supernatant, these results indicate that the main responsible molecules in the culture supernatant are IL-1 and IL-6, although a presence of other effective factors is not excluded. The anti-CD3-induced thymidine uptake by T cells in the presence of IL-1 and IL-6 was significantly inhibited by anti-Tac antibody, suggesting that the proliferation of T cells in this system is mostly mediated by a IL-2-dependent pathway. Our study further showed that accessory cells seem to acquire cell surface properties necessary for the effective interaction with T cells during 6–24 hr of culture with IFN-γ. Presumably, a certain molecule(s) required for the interaction is induced on the cell surface of the AC by IFN-γ.

INTRODUCTION

Recent success in the molecular characterization and gene cloning of various cytokines, including various interleukins, interferons (IFN) and colony-stimulating factors (CSF), have made it possible to clarify the mechanisms of various immunological phenomena at a molecular level. The effect of soluble factors on the growth and differentiation of T and B lymphocytes in immune response has been experimentally reproduced by the use of purified recombinant cytokines in many cases.

The growth of activated T cells is known to be mediated by the interaction of IL-2 with its surface receptor on T cells (Meuer *et al.*, 1984; Robb, 1984). But the molecular mechanism of the early process for triggering the T cells via antigen receptor to produce growth factor and express receptors for the growth factor has not yet been well understood. Although IL-1 has been thought to play a central role in this process (Scala & Oppenheim, 1983; Williams *et al.*, 1985; Meuer & Buschenfelde, 1986; Kaye *et al.*, 1984), observations which are apparently incompatible with such assumption have also been reported (Bekoff, Kubo & Grey, 1986; Garman & Raulet, 1987; Malek, Schmidt & Shevach, 1985; Koide, Inaba & Steinman, 1987; Mizel, 1987; Thiele, Kurosaka & Lipsky, 1983).

We recently described a system for the analysis of accessory functions essential for the induction of receptor-mediated human T-cell proliferation in which we used anti-CD3 antibody-coupled latex beads to cross-link the T-cell receptor/CD3 complex, paraformaldehyde (PFA)-fixed autologous macrophages or a monoblastic leukaemia cell line, U937, to examine the necessity of physical surface interaction with T cells and culture supernatants of macrophages or U937 cells to analyse the soluble factors required (Kawakami *et al.*, 1989). Results

Abbreviations: AC, accessory cell(s); f-Mϕ(US) and f-Mϕ(IFN-γ), unstimulated and IFN-γ-pretreated and PFA-fixed macrophages, respectively; f-U937(US) and f-U937(IFN-γ), unstimulated and IFN-γ-pretreated and PFA-fixed U937 cells, respectively; IFN-γ, interferon-gamma; PFA, paraformaldehyde; Sup, culture supernatant; αT3-L, OKT3 coupled to latex beads.

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obtained with this system indicated that the accessory cell functions are mediated by accessory cell-derived soluble factors and by physical interaction of accessory cells with T cells and both of these functions are required for the induction of IL-2 production and proliferation of T cells by anti-CD3-coupled latex beads. For physical interaction, it was found that macrophages and U937 cells had to be precultured with a culture supernatant of Con A-stimulated lymphocytes or recombinant IFN-γ before fixation with PFA. As regards the soluble factors required, we found that the culture supernatant of accessory cells could be replaced by IL-1 plus IL-6 but not by IL-1 alone. However, the factor(s) which collaborates with IL-1 in the culture supernatant remained unidentified.

In this report, we demonstrate that IL-6 acts synergistically with IL-1 to potentiate the induction of T-cell proliferation by anti-CD3 latex and that the potentiating activity of the culture supernatants is mainly ascribed to IL-1 and IL-6. The proliferation of T cells in this system seems to be mainly driven by the IL-2–IL-2 receptor system. Our study further shows that a surface molecule(s) critical for the effective interaction with T cells in this sytem appears to be induced on accessory cells after 6 hr of incubation with IFN-γ.

MATERIALS AND METHODS

Culture medium and reagents

RPMI-1640 medium was obtained from Gibco (Grand Island, NY), fetal calf serum (FCS) from Kyoto Biken Co. (Kyoto), L-leucine methylester, paraformaldehyde and glycylglycine from Nakarai Chemicals, Ltd (Kyoto) and muramyl dipeptide (MDP) for Peptide Institute Inc. (Osaka). Recombinant human BSF-2/IL-6 (Hirano *et al.*, 1985, 1986) was kindly supplied from Dr T. Hirano and Dr T. Kishimoto (Osaka University, Osaka), recombinant human IL-1β from Dr M. Hirai (Otsuka Pharmaceutical Co. Ltd, Tokushima Research Inst., Tokushina); recombinant human IL-2 and IFN-γ from Dr S. Ito (Kyowa Hakko Kogyo Co. Ltd, Tokyo Institute, Tokyo).

Cell line

A human histiocytic (monoblastic) lymphoma cell line, U937 (Sundstrom & Nilsson, 1976), was a kind gift from Dr T. Watanabe (Kyushu University, Fukuoka). It was treated with anti-mycoplasma reagent MC110 (Dainippon Pharmaceutical Co., Tokyo) and maintained in RPMI-1640 medium supplemented with 15% FCS, penicillin G (100 U/ml) and streptomycin (100 μg/ml).

Antibodies

Monoclonal antibodies, NuIa (IgG1), directed against monomorphic determinant of HLA-DR, and NuB1 (IgG1), directed against human B-cell associated antigen, were obtained from Nichirei Co. (Tokyo). A monoclonal antibody to CD3, OKT3 (IgG2a), was prepared from the culture supernatant of the OKT3 hybridoma by a protein A column. Rabbit anti-human IL-1α and IL-1β antisera were generous gifts from Dr M. Yoshinaga (Kumamoto University, Kumamoto). The antisera were obtained by immunizing rabbits with human recombinant IL-1α and IL-1β, respectively, and had the capacity to neutralize 60 U/ml IL-1α and IL-1β at 1/500 dilutions, respectively, in a thymocyte assay. Rabbit human anti-BSF-2/IL-6 antibody (Hirano *et al.*, 1988) was kindly provided by Dr T. Hirano and Dr T. Kishimoto (Osaka University, Osaka). A murine monoclonal anti-IL-2R antibody (IgG2a), anti-Tac (Uchiyama, Broder & Waldmann, 1981), was kindly provided as ascitic fluid and purified antibody by Dr T. Uchiyama (Kyoto University, Kyoto).

Purification of T cells

T cells were purified from peripheral blood mononuclear cells (PBMC) isolated from the peripheral blood of healthy adult donors by Ficoll–Hypaque density gradient centrifugation as described in the previous paper (Teranish *et al.*, 1982). Briefly, PBMC suspended at 3×10^6/ml in RPMI-1640–10% FCS supplemented with penicillin G (100 U/ml), streptomycin (100 μg/ml) and L-glutamine (2 mM) (complete medium) were incubated for 2 hr in glass dishes at 37° in 5% CO_2 air. Non-adherent cells resuspended (approximately $2\text{–}2{\cdot}5 \times 10^7$/ml) in RPMI-1640 complete medium containing 5 mM L-leucine methylester (Thiele *et al.*, 1983) applied on a nylon-wool column (1·0 ml/0·6 g nylon-wool) and nylon-wool non-adherent cells were eluted with the complete medium containing 5 mM L-leucine methylester. The cells were then suspended in RPMI-1640–5% FCS containing NuIa and NuB1 antibodies and incubated on ice for 1 hr. After washing, the cells resuspended in RPMI-1640–5% FCS were plated on dishes coated with a rabbit anti-mouse Fabγ antibody and incubated on ice for 1 hr. Unbound cells were collected and used as accessory cell-depleted T cells. The T-cell preparations thus obtained contained >96% $OKT3^+$, 81% $OKT4^+$, 17% $OKT8^+$, <1% $NuIa^+$, <1% $NuB1^+$ cells. The content of $OKT4^+$ T cells was increased as compared to the original content in PBMC, which is about 65%, probably due to the toxic effect of L-leucine methylester (Thiele *et al.*, 1983; Thiele & Lipsky, 1986).

Preparation of macrophages

PBMC suspended in RPMI-1640–10% FCS were incubated at 37° for 2 hr in glass dishes. Glass non-adherent cells were removed by five cycles of brisk agitation and washing with RPMI-1640 medium. Glass-adherent cells were dislodged with a rubber policeman, washed three times with RPMI-1640 medium and used as macrophages.

Paraformaldehyde fixation

Unstimulated macrophages and U937 cells or the cells precultured with IFN-γ (24–72 hr) were washed and fixed with 1% paraformaldehyde (PFA) in PBS for 30 min at room temperature. The fixation was stopped by washing the cells in cold 0·6% glycylglycine to block free aldehyde groups (Roska & Lipsky, 1985) and unbound paraformaldehyde was removed by several washes in RPMI-1640 medium. The fixed cells were kept in FCS-free RPMI-1640 medium at 4° until use. More than 99% of the fixed U937 cells were stained with trypan blue. It was confirmed that incubation of the PFA-fixed cells for 24 hr did not release a detectable amount of IL-1.

Preparation of OKT3 coupled to latex beads (αT3-L)

αT3-L was prepared as described previously (Fujimoto *et al.*, 1986). Briefly, 1·5 ml latex beads (Difco Lab., Detroit, MI) were coated with rabbit anti-mouse IgG2a antibody (1·28 mg in 220 μl) and the remaining uncoated surface was blocked by incubating with RPMI-1640 containing 10% FCS and suspended to the original volume of PBS. To prepare αT3-L, the

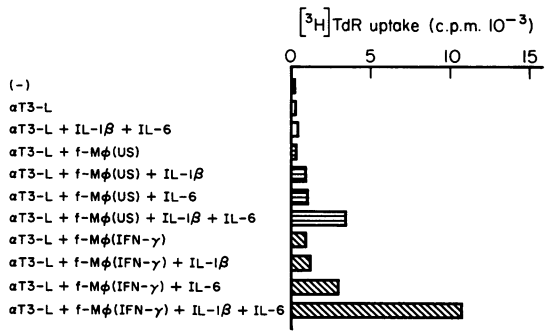

Figure 1. IL-1 and IL-6 potentiate the induction of T-cell proliferation in the presence of f-Mϕ (IFN-γ). T cells ($2{\cdot}5 \times 10^5$/ml) were cultured under the indicated combinations in 96-well microculture plate for 72 hr. The uptake of [^{3}H]thymidine during the last 8 hr was determined and expressed as means of triplicate cultures as described in the Materials and Methods. Fixed macrophages were added at 5×10^4/ml to T-cell culture. IL-1β and IL-6 were added at 10 U/ml.

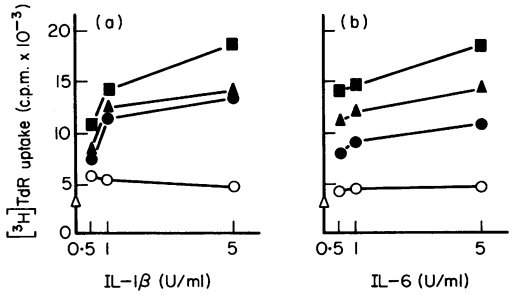

Figure 2. IL-1 and IL-6 show synergistic activity to potentiate T-cell proliferation. T cells ($2{\cdot}5 \times 10^5$/ml) were cultured with αT3-L (10%), f-U937(IFN-γ) (5×10^4/ml) in the presence of indicated doses of human recombinant IL-1β and IL-6 for 72 hr. The [^{3}H]thymidine uptake during 8 hr was determined and expressed as means of triplicate cultures as described in the Materials and Methods. (a) Human recombinant IL-6, 0·5 U/ml (●), 1 U/ml (▲), 5 U/ml (■), without IL-6 (○), without both cytokines (△). (b) Human recombinant IL-1β, 0·5 U/ml (●), 1 U/ml (▲), 5 U/ml (■), without IL-1β (○), without both cytokines (△).

latex coated with anti-mouse IgG2a antibody was incubated with a saturating amount of OKT3 antibody for 2 hr and suspended at 10% (v/v) in PBS.

Assay for T-cell proliferation

Cells were cultured in RPMI-1640–5% FCS medium supplemented with 2-mercaptoethanol (50 μM), penicillin G (100 U/ml), streptomycin (100 μg/ml) and L-glutamine (2 mM). T cells ($2{\cdot}5 \times 10^5$/ml) were cultured in 200 μl/well in flat-bottomed microtitre plates for 72 hr in the presence or absence of 20% AC (5×10^4/ml). Cultures were pulsed with 0·2 μCi of [^{3}H]thymidine for the last 8 hr and harvested by using a cell harvester, and the [^{3}H]thymidine incorporation was measured by a liquid scintillation counter. Results were expressed as mean counts per min (c.p.m.) of triplicate cultures. Standard deviations usually ranged within 10% of the mean.

Preparation of culture supernatants of macrophages

Macrophage culture supernatant (Mø Sup) was prepared by culturing the macrophages (1×10^6/ml) prepared as described above in the presence of MDP (5 μg/ml) for 2 days. The supernatants collected were dialysed against RPMI-1640 medium and sterilized by passing through a 0·22 μm filter.

RESULTS

Requirement for physical interaction with accessory cells and soluble factor for anti-CD3-induced T-cell proliferation

The experimental system we recently defined (Kawakami *et al.*, 1989) to analyse the accessory cell functions necessary for the induction of IL-2 production and proliferation of T cells was used in the present study. In this system, human peripheral blood T cells were cultured with anti-CD3 antibody coupled on latex beads (αT3-L) in the presence of PFA-fixed autologous macrophages or U937 cells and the culture supernatant of macrophages (Mϕ Sup) or U937 cells (U937 Sup). αT3-L was used to cross-link the T-cell receptor/CD3 complex.

Results in Fig. 1 show that IL-1β and IL-6, which are known to be produced by macrophages (Aarden *et al.*, 1987), act together to potentiate the induction of T-cell proliferation in this system. Recombinant IL-1β and IL-6 showed only a weak activity when used separately but these two lymphokines show much higher activity when used together than when used alone. It should be noted that both the PFA-fixed macrophages (f-Mϕ) and soluble factors are required to induce thymidine uptake of T cells by αT3-L. The results clearly show that the IFN-γ-pretreated and fixed macrophages [f-Mϕ(IFN-γ)] are much more effective than the unstimulated and fixed macrophages [f-Mϕ(US)].

Synergistic effect of IL-1 and IL-6

The effect of recombinant IL-1β and IL-6 was more quantitatively analysed in the above experimental system using f-U937 (IFN-γ) as a PFA-fixed accessory cell. As shown in Fig. 2a and b, IL-6 and IL-1β showed only a weak activity when used alone, but the thymidine uptake by T cells was remarkably increased when both were used together. The response was dependent on either one of the two lymphokines when the one was used at

Table 1. Effect of anti-IL-1 or IL-6 antibody on T-cell proliferation induced by IL-1 and IL-6

Antibodies	[^{3}H]TdR uptake (c.p.m.)
(—)	19,610
Anti-IL-1α serum (×4000 dil.)	28,762
Anti-IL-1β serum (×4000 dil.)	6959
Normal rabbit serum (×4000 dil.)	32,234
Anti-IL-6 Ab (10 μg/ml)	7758
Normal rabbit IgG (10 μg/ml)	19,786

T cells ($2{\cdot}5 \times 10^5$/ml) were cultured with αT3-L (10%), f-U937(IFN-γ) (5×10^4), IL-1β (5 U/ml) and IL-6 (5 U/ml) in the presence or absence of indicated antibodies for 72 hr. [^{3}H]Thymidine uptake during the last 8 hr of the culture was measured and expressed as means of triplicate cultures as described in the Materials and Methods. [^{3}H]Thymidine uptake in the absence of both interleukins was 5247.

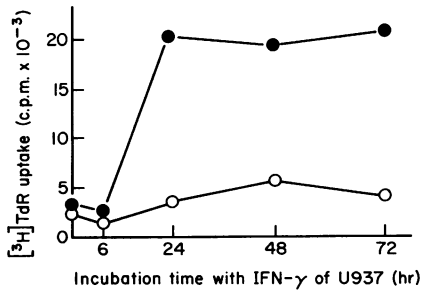

Figure 3. Time-course of IFN-γ for the acquirement of T-cell potentiating activity of U937. U937 cells were cultured with IFN-γ (500 U/ml) for indicated periods and then fixed with PFA. T cells ($2{\cdot}5 \times 10^5$/ml) were cultured with αT3-L (10%) and fixed U937 cells (5×10^4/ml) prepared as described above in the presence or absence of IL-1β (10 U/ml) and IL-6 (10 U/ml) for 72 hr. The uptake of [^{3}H]thymidine during the last 8 hr was determined and expressed as means of triplicate cultures as described in the Materials and Methods. With IL-1β and IL-6 (●), without both cytokines (○).

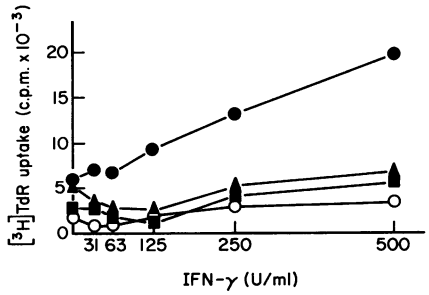

Figure 4. Dose effect of IFN-γ for the acquirement of T-cell potentiating activity of U937. U937 cells were cultured with indicated doses of IFN-γ for 2 days and then fixed with PFA. T cells ($2{\cdot}5 \times 10^5$/ml) were cultured with αT3-L (10%) and fixed U937 cells (5×10^4/ml) prepared as described above in the presence or absence of IL-1 (10 U/ml) or / and IL-6 (10 U/ml) for 72 hr. The uptake of [^{3}H]thymidine during the last 8 hr was determined and expressed as means of triplicate cultures as described in the Materials and Methods. With IL-1β and IL-6 (●), IL-1β (▲), IL-6 (■), without both cytokines (○).

various concentrations together with a constant dose of the other. The maximum thymidine uptake in these experiments reached about 90% of that shown in the presence of live macrophages instead of f-U937(IFN-γ) and the two lymphokines. The effect of U937 cells was not due to its allogenic effect because addition of f-U937(US) or f-U937(IFN-γ) without the two lymphokines did not show any significant potentiating activity. As shown in Table 1, the effect of combined addition of IL-1β and IL-6 on T-cell proliferation was almost completely inhibited to the level of thymidine uptake in the absence of both interleukins by either anti-IL-1β serum or anti-IL-6 antibody (IgG fraction of antiserum) but not by anti-IL-1α serum, control normal serum or control normal IgG.

These results indicate that IL-1β and IL-6 act synergistically to potentiate the induction of T-cell proliferation in this experimental system.

Time-course and dose effect of IFN-γ for the acquirement of surface-interacting competence of U937 cells

U937 cells or macrophages had to be precultured with IFN-γ before fixation with PFA to reveal a potentiating effect for αT3-L-induced T-cell proliferation in the presence of IL-1β plus IL-6.

The results depicted in Fig. 3 show that the potentiating competence of U937 cells was acquired some time between 6 and 24 hr after the initiation of the culture of U937 cells with IFN-γ. The dose effect of IFN-γ to affect the potentiating competence of U937 cells is shown in Fig. 4. The potentiating capacity of fixed U937 cells increased as the dose of IFN-γ used for preculture was increased and did not reach the maximum at 500 U/ml of IFN-γ tested.

Effect of anti-IL-2 receptor antibody on the αT3-L-induced T-cell proliferation

Next, the effect of a monoclonal anti-IL-2 receptor antibody, anti-Tac, on the αT3-L-induced proliferation of T cells was examined. As shown in Fig. 5, addition of anti-Tac antibody inhibited the thymidine uptake of T cells induced by αT3-L in the presence of f-U937(IFN-γ) and IL-1β plus IL-6. The response was significantly inhibited at 1:1000–1:100 dilution of the anti-Tac ascitic fluid or 5–50 μg/ml purified antibody, whereas no inhibition was observed with control ascitic fluid at the same dilution. The addition of 2000 U/ml rIL-2 completely reversed the effects of 1:1000 dilution of ascitic fluid or 5 μg/ml of anti-Tac antibody but only partially those of 1:100 dilution or 50 μg/ml antibody. In a control experiment carried out to show the inhibiting effect of the anti-Tac antibody on IL-2-dependent growth, the αT3-L-induced proliferation of T cells in the presence of recombinant IL-2 was efficiently inhibited at lower concentrations of the antibody (data not shown).

Inhibition of the potentiating activity of macrophage culture supernatant (Mϕ Sup) by anti-IL-1 and anti-IL-6 antibodies

To obtain evidence to show that IL-6 exists in Mϕ Sup and acts to potentiate αT3-L-induced T-cell proliferation, the effect

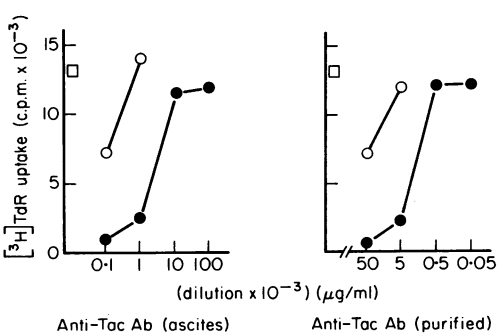

Figure 5. Effect of anti-Tac antibody on the induction of T-cell proliferation. T cells ($2{\cdot}5 \times 10^5$/ml) were cultured with αT3-L (10%), f-U937(IFN-γ) (5×10^4/ml) and IL-1β (10 U/ml) plus IL-6 (10 U/ml) in the presence or absence of anti-Tac antibody (ascitic fluid or purified antibody) or anti-Tac antibody plus excess dose (2000 U/ml) of IL-2. The uptake of [^{3}H]thymidine during the last 8 hr of 72-hr culture was determined and expressed as means of triplicate cultures, as described in the Materials and Methods. Without antibody (□), with anti-Tac antibody (●), anti-Tac antibody and IL-2 (○).

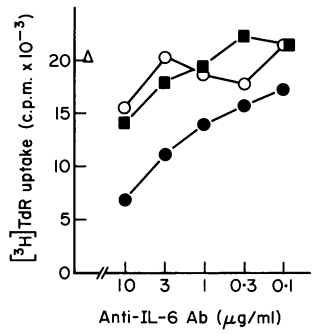

Figure 6. Anti-IL-6 antibody inhibits the potentiating activity of Mϕ Sup on αT3-L-induced T-cell proliferation. T cells ($2{\cdot}5 \times 10^5$/ml) were cultured with αT3-L (10%), f-U937(IFN-γ) (5×10^4/ml) and Mϕ Sup (6·25%) in the presence or absence of anti-IL-6 antibody, normal rabbit IgG or anti-IL-6 antibody and excess dose (200 U/ml) of IL-6. The uptake of [^{3}H]thymidine during the last 8 hr of 72-hr culture was determined and expressed as means of triplicate cultures as described in Materials and Methods. Normal rabbit IgG (■), anti-IL-6 antibody (●), anti-IL-6 antibody and IL-6 (○), without antibody (△).

of anti-IL-6 antibody was tested. Results shown in Fig. 6 indicate that at 0·1–3·0 μg/ml of anti-IL-6 antibody (IgG fraction), the αT3-L-induced thymidine uptake in the presence of Mϕ Sup and f-U937(IFN-γ) was dose-dependently inhibited by this antibody, whereas normal rabbit IgG used as a control showed no inhibition at this range of concentration. At a higher dose (10 μg/ml), the effect of Mϕ Sup was completely inhibited by the antibody to the level observed in the absence of Mϕ Sup. At this concentration, however, normal rabbit IgG also showed some inhibitory effect. Nevertheless, large differences can be seen in the degree of inhibition between anti-IL-6 antibody and control IgG. Furthermore, addition of an excess dose of recombinant IL-6 completely reversed the thymidine uptake inhibited by anti-IL-6 antibody to the level in the presence of control IgG. These results, together with our recently obtained results (Kawakami *et al.*, 1989), that the potentiating activity of Mϕ Sup for αT3-L-induced T-cell proliferation was dose-dependently inhibited by the addition of rabbit anti-human IL-1β serum, give edivence to support the view that IL-1β and IL-6 present in Mϕ Sup are acting synergistically to potentiate the αT3-L-induced T-cell proliferation.

DISCUSSION

The present study demonstrated that recombinant IL-1 in combination with IL-6 supports the induction of human T-cell proliferation mediated by anti-CD3 coupled on latex beads. The two interleukins act synergistically so that the potentiating activity was substantially reduced by antibody to either IL-1 or IL-6. This finding would provide a reason for the observed ineffectiveness of IL-1 to support the induction of T-cell proliferation in some cases (Bekoff *et al.*, 1986; Garman & Raulet, 1987). Lack of the partner factor may result in the apparent lack of the activity.

Our data show that the potentiating activity of Mϕ Sup was largely inhibited by anti-IL-6. In conjunction with the result we recently reported (Kawakami *et al.*, 1989), that anti-IL-1 strongly inhibited the potentiating activity of Mϕ Sup, it seems that the main effective potentiating factors released from macrophages are IL-1 and IL-6, although the presence of other factors is not excluded.

B-cell stimulating factor 2 (BSF-2)/IL-6, originally isolated and cloned as a B-cell differentiation factor (Hirano *et al.*, 1985, 1986), was found to be identical with several factors such as hybridoma growth factor (Brakenhoff *et al.*, 1987), hepatocyte-stimulating factor (Gauldie *et al.*, 1987) and interferon-β2 (Sehgal *et al.*, 1987) and was shown to have a wide variety of biological activities (Wong & Clark, 1988). The effectiveness of IL-6 to act as a co-stimulant for T-cell activation was reported recently by Garman *et al.* (1987) for murine T cells and by Lotz *et al.* (1988) for human T cells and thymocytes. More recently, Houssiau *et al.* (1988) reported that IL-1 and IL-6 act synergistically to potentiate the proliferation of purified human tonsillar T cells by PHA stimulation.

Results of ours and of Houssiau *et al.* (1988) are different from those of Garman *et al.* (1987) and Lotz *et al.* (1988) in that the former groups showed that induction of PHA- or anti-TcR-mediated T-cell proliferation was potentiated by synergistic action of IL-1 and IL-6, whereas the co-stimulant activity was observed by IL-6 alone in the studies of the latter groups. A possibility which might be suggested is that this difference could be due to incompleteness of the removal of accessory cells which might obscure the requirement of IL-1 and IL-6 for synergistic action.

An important finding in our study is that the anti-CD3-mediated T-cell proliferation can only be induced when the two interleukins, IL-1 and IL-6, are supplied along with the IFN-γ-pretreated and PFA-fixed macrophages [f-Mϕ(IFN-γ)] or U937 cells [f-U937(IFN-γ)]. Our results indicate the requirement for physical and factor-mediated interaction of accessory cells with T cells to activate and induce proliferation of T cells. The necessity of the preculture of accessory cells with IFN-γ before PFA-fixation was clearly shown, suggesting an involvement of IFN-γ-inducible surface molecule(s) in the accessory cell–T-cell interaction. However, the necessity of physical interaction was not shown in the study of Houssiau *et al.* (1988) and the more recent study by Tosato & Pike (1988). The reason for this discrepancy is not known at present, but it suggests that the triggering of T cells by PHA is not the same as that by anti-CD3 antibody. In fact, it is reported that lectins bind to T-cell receptors as well as other surface molecules (Sitkovsky *et al.*, 1984; O'Flynn *et al.*, 1985; Bernabeau *et al.*, 1987) and some of these interactions may create effective signals which replace or bypass the signal transduction created by physical interaction between accessory cells and T cells. Determination of the surface molecules involved in such cellular interaction will help to solve the problem.

The anti-CD3-mediated T-cell proliferation induced by Mϕ Sup or IL-1 and IL-6 in the presence of f-U937(IFN-γ) was inhibited by anti-IL-2R (anti-Tac) antibody. Although the inhibition was seen at a relatively high concentration of the antibody (5 μg/ml), the inhibition seems to be specific since the effect at this concentration of antibody was completely reversed by the addition of excess dose of recombinant IL-2. Requirement for high concentration of anti-IL-2 receptor antibody seems to be consistent with the result reported by Lotz *et al.* (1988), suggesting that the proliferation may not be exclusively dependent on the IL-2/IL-2R pathway. On the other hand, Tosato & Pike (1988) have reported that T-cell proliferation induced by PHA and IL-6 was not blocked by anti-IL-2R

antibody. The reason for this apparent discrepancy is not known. There are reports showing the evidence for IL-2-independent proliferation of T cells (Moldwin *et al.*, 1986; Laing & Weiss, 1988). However, it is not known in the present study whether some residual thymidine uptake in the presence of a high concentration of anti-IL-2R antibody is due to the presence of a pathway other than the IL-2/IL-2R route or not.

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