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Development of the cell contact-mediated accessory function for T-cell proliferation in a human promyelocytic leukaemia cell line, HL-60, by 1,25-dihydroxyvitamin D_3

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SUMMARY

A human promyelocytic leukaemia cell line, HL-60 cells, did not show accessory cell (AC) function to potentiate the proliferation of human T cells induced by anti-CD3 antibody coupled to latex beads (αT3-L). This was found to be at least due to the inability of HL-60 cells to express certain molecules which are inducible with interferon-gamma (IFN-γ) on mature monocytes and are necessary for interaction with T cells. HL-60 cells acquired the ability to express such surface molecules by stimulation with IFN-γ when the cells were pretreated with 1,25-dihydroxyvitamin D_3 (Vit D). The effect of Vit D was reversible, that is, the AC function of the HL-60 cells was lost when the cells were cultured in Vit D-free medium for 7 days. It was also found that HL-60 cells treated with IFN-γ and then with Vit D did not show significant AC function. The flow cytometric analysis showed that the expression of HLA-DR and intercellular adhesion molecule-1 (ICAM-1) was highly increased on HL-60 cells when stimulated with IFN-γ after treatment with Vit D. The expression of ICAM-1 was also induced with IFN-γ on untreated cells but in lower amounts. Monoclonal antibodies against ICAM-1 and HLA-DR inhibited the αT3-L-induced T-cell proliferation, indicating that these molecules are at least required for contact-mediated AC function. Thus our study revealed that HL-60 cells express cell surface interaction molecules necessary for potentiating the T-cell proliferation through two steps, differentiation with Vit D to mature monocyte-like cells followed by stimulation with IFN-γ.

INTRODUCTION

It has been reported that for successful induction of T-cell proliferation, not only the binding of the T-cell receptor–CD3 (TcR/CD3) complex with the antigen–major histocompatibility complex (MHC) but also the auxiliary signals mediated by soluble factors released from accessory cells (AC) and cell-to-cell interactions between T cells and AC are required.[1–3] In T-cell activation, cell surface interaction molecules are considered to reinforce or stabilize the physical interaction of T cells and AC. In addition, interaction of some surface molecules such as CD2 or lymphocyte function-associated antigen-1 (LFA-1) seem to deliver signals which co-stimulate T cells.[4–6]

Abbreviations: AC, accessory cell; αT3-L, OKT3 coupled to latex beads; IFN-γ, interferon-gamma; mAb, monoclonal antibody; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cell; PFA, paraformaldehyde; Vit D, 1,25-dihydroxyvitamin D_3.

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Using a model system for the CD3-monoclonal antibody-mediated T-cell proliferation, our previous studies[7,8] demonstrated that optimal T-cell activation requires cross-linking of the TcR/CD3 complex, co-stimulatory signals mediated by cell surface interaction between T cells and monocytes, and soluble factors, interleukin-1 beta (IL-1β) and IL-6, released from monocytes. Of particular interest was the finding that certain surface molecule(s) induced by interferon-gamma (IFN-γ) during the T-cell–monocyte interaction was essential for this cell contact-mediated co-stimulation. Thus, the paraformaldehyde (PFA)-fixed IFN-γ-stimulated monocytes but not the PFA-fixed unstimulated monocytes were found to be capable of mediating the effective cell-to-cell interaction to induce T-cell proliferation in the presence of IL-1β and IL-6.

To clarify the molecular mechanisms involved in contact-mediated accessory function, it should be of value to study how this function develops during the differentiation of immature monocytes and compare the surface molecules as well as the intracellular signal transducing system of immature and matured cells. Along this line, we examined in this study the ability of HL-60 cells to express cell contact-mediated accessory

function. Our study demonstrated that the ability could be developed by *in vitro* treatment of HL-60 cells with 1,25-dihydroxyvitamin D_3 (Vit D) and the ability is largely dependent on the capacity of cells to express critical cell-interaction molecules in response to IFN-γ.

MATERIALS AND METHODS

Culture medium and reagents

RPMI-1640 medium was obtained from Gibco (Grand Island, NY), foetal calf serum (FCS) from Kyoto Biken Co. (Kyoto, Japan), L-leucine methylester, PFA and glycylglycine from Nakarai Chemicals, Ltd (Kyoto, Japan) and Vit D from Biomol Res. Lab. Inc. (Plymouth Meeting, PA). Human recombinant IL-1β was a generous gift from Dr M. Hirai (Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan) and human recombinant IL-6[9,10] was kindly provided by Drs T. Hirano and T. Kishimoto of Osaka University, Japan. Human recombinant IFN-γ was a kind gift from Dr S. Ito (Kyowa Hakko Kogyo Co., Ltd, Tokyo Institute, Tokyo, Japan) and was also purchased from Collaborative Research Inc. (Bedford, MA).

Cell lines

A human histiocytic (monoblastic) lymphoma cell line, U937,[11] was a kind gift from Dr T. Watanabe of Kyushu University, Japan. A human promyelocytic cell line, HL-60[12] was a kind gift from Dr Hirashima of Kumamoto University, Japan. They were treated with anti-mycoplasma reagent MC110 (Dainippon Pharmaceutical Co., Tokyo, Japan) and maintained in RPMI-1640 medium supplemented with 15% FCS, penicillin G (100 U/ml) and streptomycin (100 μg/ml).

Antibodies

Monoclonal antibodies (mAb), NuIa (IgG1), directed against monomorphic determinant of HLA-DR, and NuB2 (IgG2b), directed against CD20 were obtained from Nichirei Co. (Tokyo, Japan). A mAb, HLA-DR (IgG2a), directed against monomorphic determinant of HLA-DR was obtained from Becton Dickinson Immunocytometry Systems (San Jose, CA). Fluorescein isothiocynate (FITC)-labelled $F(ab')_2$ fragments of sheep anti-mouse IgG were obtained from Cappel Laboratories (West Chester, PA). Monoclonal antibodies to CD3 (OKT3, IgG2a) and to CD4 (OKT4, IgG2b) were prepared by a protein A column from the culture supernatant of the OKT3 hybridoma and from the ascites of the OKT4 hybridoma-transplanted mice, respectively. A mAb, TS1/22.1.1.13 (IgG1), directed against α-subunit of LFA-1 was also prepared by a protein A column from the culture supernatant of the TS1/22.1.1.13 hybridoma. A mAb, HA58 (IgG1),[13] directed against intercellular adhesion molecule-1 (ICAM-1) was kindly supplied from Drs H. Hirata and K. Imai of Sapporo Medical College, Japan, and BB-1 (IgM)[14] directed against activated B-cell antigen (B7/BB-1) from Dr T. Yokochi of Aichi Medical College, Japan.

Purification of T cells

T cells were purified from peripheral blood mononuclear cells (PBMC) of healthy adult donors by Ficoll–Hypaque density gradient centrifugation as described previously.[15] 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_2–95% air. Non-adherent cells resuspended (approximately $2–2{\cdot}5 \times 10^7$/ml) in RPMI-1640 complete medium containing 5 mM L-leucine methylester[16] were 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 NuB2 antibodies and incubated on ice for 1 hr. After washing, the cells resuspended in RPMI-1640–5% FCS were plated on a dish coated with a rabbit anti-mouse Fabγ antibody and incubated on ice for 1 hr. Unbound cells were collected and used as AC-depleted T cells. The T-cell preparations thus obtained contained >96% $OKT3^+$, 82% $OKT4^+$, 13% $OKT8^+$, <1% $NuIa^+$, <1% $NuB2^+$ cells. The content of $OKT4^+$ T cells was increased compared to the original content in PBMC, which is about 62%, probably due to the toxic effect against $OKT8^+$ T cells of L-leucine methylester.[16,17]

Preparation of monocytes

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 monocytes.

Treatment of HL-60 cells with Vit D and IFN-γ

HL-60 cells were cultured at 1×10^5/ml in Petri dishes (Falcon 1029) with 1×10^{-7} M Vit D for 4 days at standard conditions. Then the cells were washed and further cultured with 500 U/ml recombinant IFN-γ for 2 days. The cells were irradiated or fixed with PFA and used as AC.

In order to minimize the contamination of water with lipopolysaccharide (LPS), the water prepared by the Milli Q system was further distilled before use. The LPS content of Vit D solution at 1×10^{-6} M (10 times the concentration used in most of the experiments) was 4–5 pg/ml as determined by limulus assay, which was the same level as that of water.

Paraformaldehyde fixation

Unstimulated or pretreated HL-60 cells were washed and fixed with 1% PFA in phosphate-buffered saline (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[18] and unbound PFA 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 96% of the fixed HL-60 cells were stained with trypan blue.

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

αT3-L was prepared as described previously.[19] Briefly, 1·5 ml latex beads (Difco, 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 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

T cells ($2{\cdot}5 \times 10^5$/ml) were cultured in RPMI-1640–10% FCS medium supplemented with 2-mercaptoethanol (50 μM), penicillin G (100 U/ml), streptomycin (100 μg/ml) and L-glutamine (2 mM) 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 the cells were 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.

Immunofluorescence staining

Cell suspensions of the HL-60 cells unstimulated or treated in various ways were incubated with normal mouse IgG (5–10 μg/ml), HLA-DR (5–10 μg/ml) or HA58 (5–10 μg/ml) in the presence of human IgG (500–1500 μg/ml) for 30 min at 4°. The cells were washed twice with PBS, further incubated with FITC-labelled F(ab′)$_2$ fragments of sheep anti-mouse IgG for 30 min at 4°. After washing, the cells were analysed by Epics Profile (Coulter Corp., Hialeah, FL).

RESULTS

Effect of Vit D on co-stimulatory function of HL-60 cells

Irradiated U937 cells potentiated the αT3-L-mediated T-cell proliferation (data not shown). On the other hand, as shown in Table 1 the more immature promyelocytic leukaemia cell line, HL-60 cells, did not show this AC function. However, when HL-60 cells were cultured with Vit D for 4 days, the cells acquired the activity to potentiate the αT3-L-mediated T-cell proliferation (Table 1).

The thymidine uptake of T cells observed was not due to allogeneic reaction against the irradiated HL-60 cells because no significantly increased thymidine uptake was observed in a mixed culture for 3 days of T cells with irradiated HL-60 cells irrespective of the preculture with Vit D or not (Table 1, Exp. 1).

Table 1. Irradiated HL-60 cells precultured with Vit D potentiated anti-CD3-mediated T-cell proliferation

	[^{3}H]TdR uptake (c.p.m.)
Exp. 1	
T + αT3-Ab + irradiated HL-60	274
T + αT3-Ab + irradiated HL-60 (Vit D)	54,969
T + irradiated HL-60	169
T + irradiated HL-60 (Vit D)	237
Exp. 2	
T	96
T + αT3-L	331
T + αT3-L + irradiated HL-60	3301
T + αT3-L + irradiated HL-60 (Vit D)	29,092
Irradiated HL-60	218
Irradiated HL-60 (Vit D)	97

T cells ($2{\cdot}5 \times 10^5$/ml) were cultured with or without soluble anti-CD3 antibody (αT3-Ab), 30 ng/ml, in Exp. 1 or anti-CD3 antibody coupled to latex beads (αT3-L), 10%, in Exp. 2 for 72 hr. The [^{3}H]thymidine uptake during the last 8 hr was determined and expressed as means of triplicate cultures as described in the Materials and Methods. HL-60 cells untreated or precultured with 1×10^{-7} M Vit D for 4 days without stimulation with IFN-γ were irradiated (4500 rads) and added at 5×10^4/ml to T-cell culture.

Lack of contact-mediated AC function of HL-60 cells and its acquirement by Vit D treatment

In order to analyse whether HL-60 cells lack the ability of contact-mediated AC function for T-cell proliferation, we examined the cell-interaction activity of PFA-fixed HL-60 cells previously treated with Vit D, IFN-γ or untreated. Human peripheral blood T cells were cultured with αT3-L in the presence of PFA-fixed HL-60 cells which were treated or untreated, with or without human recombinant IL-1β and IL-6. The results depicted in Fig. 1 (Exp. 1) show that the fixed HL-60 cells which were unstimulated [f-HL-60 (US)] or precultured with Vit D for 4 days prior to fixation [f-HL-60 (Vit D)] did not potentiate the αT3-L-mediated T-cell proliferation even in the presence of recombinant IL-1β and IL-6. Fixed HL-60 cells previously stimulated with IFN-γ for 2 days [f-HL-60 (IFN-γ)] only slightly potentiated the αT3-L-mediated T-cell proliferation in the presence of recombinant IL-1β and IL-6. On the other hand, the fixed Vit D-precultured and IFN-γ-stimulated HL-60 cells [f-HL-60 (Vit D-IFN-γ)] clearly potentiated the αT3-L-mediated T-cell proliferation in the presence of recombinant IL-1β and IL-6. IL-1β and IL-6 are both required for the proliferation of T cells to the level of the positive control, in which live peripheral monocytes were used as AC. The effect of cell surface interaction of f-HL-60 (Vit D–IFN-γ) and that of exogeneously added lymphokines were synergistic because f-HL-60 (Vit D–IFN-γ) without the interleukins or the interleukins with ineffective f-HL-60 (US) or f-HL-60 (Vit D) did not potentiate the αT3-L-mediated T-cell proliferation.

Since no increased thymidine uptake was observed in a mixed culture for 3 days of T cells and f-HL-60 (Vit D–IFN-γ) in the presence of human recombinant IL-1β and IL-6 (Fig. 1, Exp. 2), the thymidine uptake of T cells was not due to allogeneic reaction against f-HL-60 (Vit D–IFN-γ). When HL-60 cells were first stimulated with IFN-γ and subsequently treated with Vit D [f-HL-60 (IFN-γ-Vit D)], the induction of contact-mediated AC function was only minimal (Fig. 1, Exp. 2).

Dose effect and time–course of Vit D necessary for acquirement of contact-mediated accessory function of HL-60 cells

HL-60 cells were treated with Vit D at various concentrations and for various periods of time, stimulated with IFN-γ and fixed with PFA and the accessory function for αT3-L-mediated T-cell proliferation was tested in the presence of recombinant IL-1β and IL-6. We previously examined the dose effect and time–course of the effect of IFN-γ on the induction of AC function in U937 cells,[8] and the optimal conditions (500 U/ml, 48 hr) were chosen from that data for the treatment of HL-60 cells in these experiments. As shown in Fig. 2, the contact-mediated AC function was induced when cultured with Vit D at concentrations higher than 1×10^{-7} M (Fig. 2a) and optimal differentiation was almost reached at 4 days of culture (Fig. 2b).

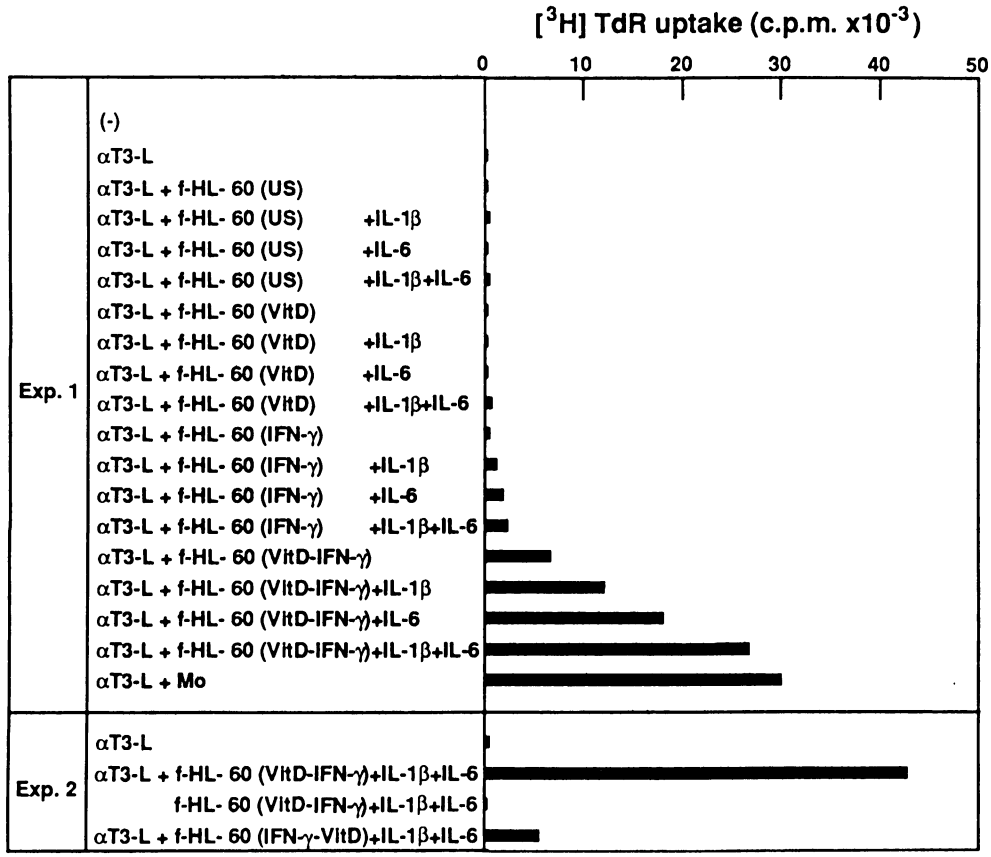

Figure 1. Contact-mediated AC function of PFA-fixed HL-60 cells previously treated or untreated with Vit D. T cells ($2{\cdot}5 \times 10^5$/ml) were cultured under the indicated combinations of cells and interleukins for 72 hr. The uptake of [^{3}H]thymidine during the last 8 hr was determined as described in the Materials and Methods. Vit D was used at 1×10^{-7} M for 4 days and IFN-γ at 500 U/ml for 2 days to differentiate and stimulate HL-60 cells. Fixed HL-60 cells and live monocytes were added at 5×10^4/ml to T-cell culture. Human recombinant IL-1β and IL-6 were added at 10 U/ml.

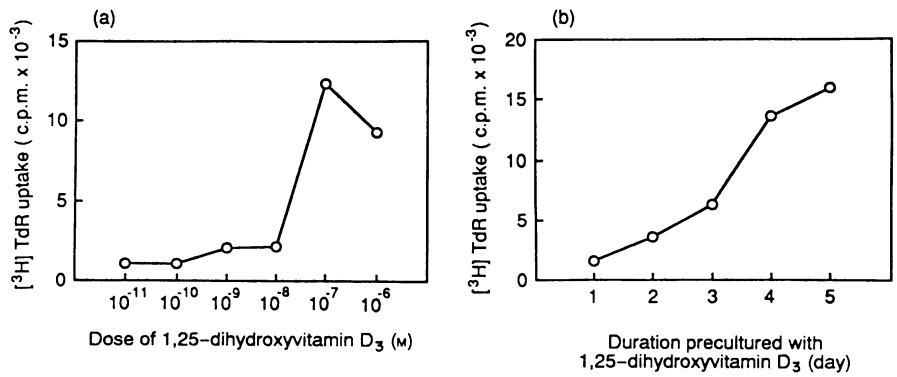

Figure 2. Dose-dependent effect of Vit D on the appearance of contact-mediated accessory function of HL-60 cells and its time-course. T cells ($2{\cdot}5 \times 10^5$/ml) were cultured with αT3-L (10%) and fixed HL-60 cells (5×10^4/ml) in the presence of human recombinant IL-1β (10 U/ml) and IL-6 (10 U/ml) for 72 hr. The [^{3}H]thymidine uptake during the last 8 hr was determined as described in the Materials and Methods. (a) HL-60 cells were precultured with various concentrations of Vit D for 4 days, stimulated with 500 U/ml IFN-γ and fixed with PFA as described in the Materials and Methods. (b) HL-60 cells were precultured with 1×10^{-7} M Vit D for various periods of time, followed by the same treatment described in (a).

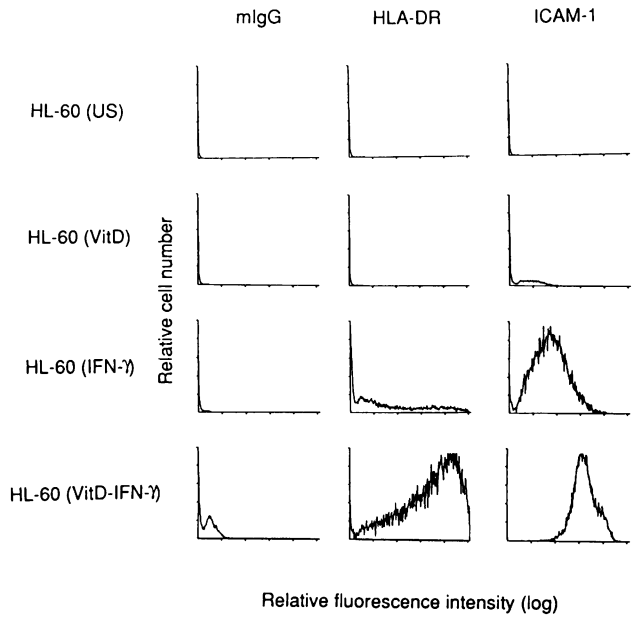

Figure 3. Expression of HLA-DR and ICAM-1 on HL-60 cells. HL-60 cells untreated, differentiated with 1×10^{-7} M Vit D for 4 days, stimulated with IFN-γ for 2 days, or differentiated with Vit D for 4 days and subsequently stimulated with IFN-γ for 2 days were stained with either anti-HLA-DR mAb or anti-ICAM-1 mAb or control mouse IgG as described in the Materials and Methods and analysed by flow cytometry.

The effect of Vit D was transient because HL-60 cells which were precultured with Vit D for 4 or 5 days and further cultured in Vit D-free medium for 7 days or more and fixed with PFA after stimulation with IFN-γ did not show the co-stimulatory activity for the αT3-L-mediated T-cell proliferation in the presence of human recombinant IL-1β and IL-6 (data not shown).

Change in expression of cell surface interaction molecules on HL-60 cells along with the differentiation with Vit D and stimulation with IFN-γ

Next, we examined the expression of surface molecules on HL-60 cells which are thought to be important for cell-to-cell interaction. As shown in Fig. 3, the MHC class II, HLA-DR, was not expressed on unstimulated or Vit D-differentiated HL-60 cells. ICAM-1 was not expressed on unstimulated HL-60 cells, but slightly expressed on Vit D-differentiated HL-60 cells. The undifferentiated HL-60 cells stimulated with IFN-γ expressed HLA-DR only slightly but significantly expressed ICAM-1. However, when the HL-60 cells were differentiated with Vit D prior to stimulation with IFN-γ, the expression of HLA-DR and ICAM-1 was dramatically augmented.

Another MHC class II protein, HLA-DQ, and B7/BB-1, which are known to be expressed on activated B cells[14,20] and IFN-γ-stimulated monocytes[21] and to be one of the ligand(s) of CD28,[22] were not expressed on HL-60 cells even when stimulated with IFN-γ after differentiation with Vit D (data not shown).

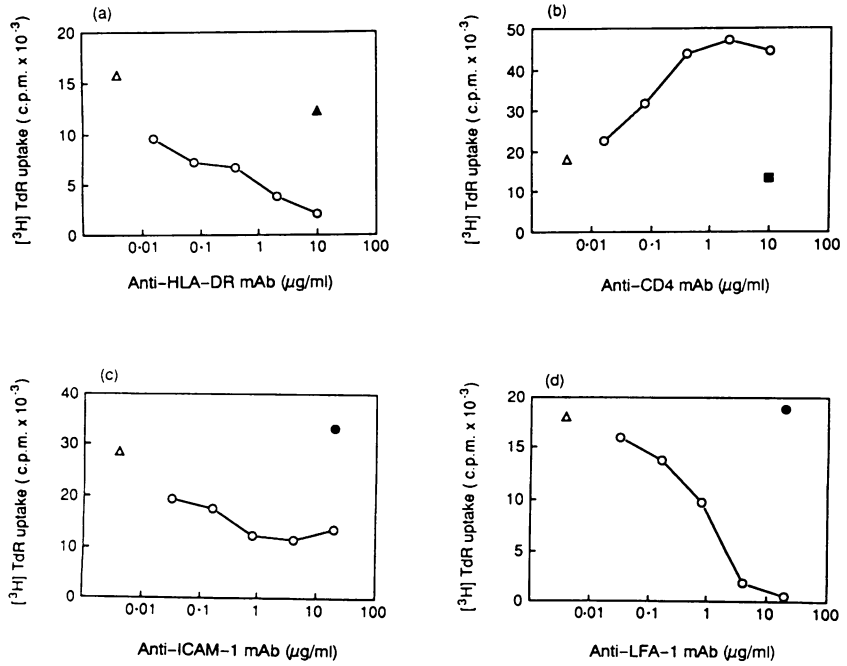

Figure 4. Effect of monoclonal antibodies against ICAM-1, LFA-1, HLA-DR and CD4 on αT3-L-induced T-cell proliferation. T cells ($2{\cdot}5 \times 10^5$/ml) were cultured with αT3-L (10%), f-HL-60 (Vit D–IFN-γ) (5×10^4/ml), human recombinant IL-1β (10 U/ml) and IL-6 (10 U/ml) without antibody or in the presence of mAb or control mouse IgG at indicated doses for 72 hr. The [^{3}H]thymidine uptake during the last 8 hr was determined as described in the Materials and Methods. (a) HLA-DR (○——○), normal mouse IgG2a (▲), no antibody (△). (b) OKT4 (○——○), normal mouse IgG2b (■), no antibody (△). (c) HA58 (○——○), normal mouse IgG1 (●), no antibody (△). (d) TS1/22.1.1.13 (○——○), normal mouse IgG1 (●), no antibody (△).

Effect of monoclonal antibodies against HLA-DR, ICAM-1 and LFA-1 on αT3-L-induced T-cell proliferation

The mAb against HLA-DR (Fig. 4a), ICAM-1 (Fig. 4c), and α-subunit of LFA-1 (Fig. 4d) all inhibited the αT3-L-induced T-cell proliferation in a dose-dependent manner, although the inhibition by anti-ICAM-1 mAb was partial. When HL-60 cells differentiated by Vit D and stimulated with IFN-γ were incubated with anti-HLA-DR mAb prior to PFA fixation, the AC function of f-HL-60 (Vit D–IFN-γ) for the αT3-L-mediated T-cell proliferation was dose-dependently inhibited by this antibody (data not shown). An mAb against CD4, which is one of the counter partner(s) of HLA-DR, augmented the αT3-L-induced T-cell proliferation (Fig. 4b).

DISCUSSION

Using immature promyelocytic HL-60 cells, we demonstrated that these cells acquire the cell contact-mediated accessory function for anti-CD3 antibody-mediated T-cell proliferation by treatment with Vit D. The development of the ability was shown to be due to the acquisition of the capacity to express certain critical cell-interaction surface molecules in response to IFN-γ.

We first showed that irradiated Vit D-treated HL-60 cells had co-stimulatory function for the anti-CD3-mediated T-cell proliferation. Next we examined the contact-mediated AC function of HL-60 cells using the model system we previously reported[7,8] in which the accessory function of monocytes for T-cell proliferation was analysed by adding PFA-fixed monocytes to the culture of T cells with αT3-L, and soluble factors, recombinant human IL-1β and IL-6. It was revealed in this system that PFA-fixed IFN-γ-stimulated HL-60 cells could not show contact-mediated accessory activity, whereas PFA-fixed Vit D-treated and IFN-γ-stimulated cells did show the AC function. In contrast, when HL-60 cells were first stimulated with IFN-γ and then treated with Vit D, no significant AC function was induced, indicating that HL-60 cells do not have the ability to express necessary surface interaction molecules in response to IFN-γ and the cells acquire this ability as they differentiate by treatment with Vit D.

The effect of Vit D was transient because the HL-60 cells which acquired the competence for AC function by treatment with Vit D became incompetent when cultured for 7 days or more without Vit D. This suggests that the tumour cell line, HL-60 cells, cannot be permanently differentiated at least by Vit D alone.

Along with the differentiation of HL-60 cells with Vit D, changes in the expression of surface molecules, particularly in the expression of molecules inducible by IFN-γ, were observed. Undifferentiated HL-60 cells did not express HLA-DR and ICAM-1. The expression of these molecules was dramatically increased when the cells were differentiated with Vit D and stimulated with IFN-γ. Conversely, when the cells were treated first with IFN-γ and then with Vit D, HLA-DR was not expressed and ICAM-1 was only slightly expressed (data not shown). Since antibodies against HLA-DR and ICAM-1 inhibited the T-cell proliferation, at least these molecules would be involved in cell interaction necessary to induce T-cell proliferation. Thus, the increased capacity to express HLA-DR and ICAM-1 may be one of the reasons for the acquisition of the AC function by HL-60 cells upon differentiation with Vit D.

MHC class II molecules carry a critical role in the antigen-specific T-cell activation and proliferation.[23,24] Furthermore, MHC class II molecules also seem to be involved in polyclonal anti-CD3-induced T-cell proliferation because mAb against MHC class II molecules inhibit monocyte-dependent anti-CD3-induced T-cell proliferation.[25,26] Racioppi *et al.* reported that the mRNA levels of IL-1β and IL-6 induced in monocytes cultured with T cells in the presence of anti-CD3 antibody were decreased by mAb against MHC class II molecules.[27] Ruggiero *et al.* also reported that MHC class II molecules on monocytes regulate the anti-CD3-induced T-cell proliferation through physical interaction.[28] In our study the anti-HLA-DR mAb bound on HLA-DR on differentiated and IFN-γ-stimulated HL-60 cells prior to PFA fixation inhibited T-cell proliferation induced by αT3-L, confirming the role of HLA-DR molecules in physical interaction for T-cell activation.

In general, it has been reported that cross-linking the CD4 molecules on the surface of T cells in conjunction with the cross-linking of TcR/CD3 complex leads to an increased T-cell proliferation, but soluble anti-CD4 mAb inhibit T-cell proliferation.[29] However, the effect of anti-CD4 mAb to T-cell proliferation is the subject of controversy. For example, there are also a few reports that soluble anti-CD4 mAb augment T-cell proliferation.[30,31] One possible explanation for the augmentation of anti-CD3-mediated T-cell proliferation by OKT4 mAb observed in our experiment may be that OKT4 mAb bound on AC through the Fc receptors cross-linked CD4 molecules on T cells and positive signals were delivered to T cells.

Although it was revealed that expression of HLA-DR and ICAM-1 on HL-60 cells was important for contact-mediated AC function, we do not necessarily argue that the expression of HLA-DR and ICAM-1 on AC are enough to potentiate T-cell activation. Certain other surface interaction molecules could have additively or synergistically potentiated the T-cell activation. Analysis of the development of accessory function in HL-60 cells will be very useful to further clarify dynamic cell interaction of AC and T cells at the molecular level.

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