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Up-regulation of ICAM-1 expression on human dermal fibroblasts by IFN-β in the presence of TNF-α

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Abstract Unstimulated human fibroblasts show low or undetectable ICAM-1 expression. Interferon-beta (IFN-β) at concentrations of 10, 100, and 1000 IU/ml in the presence of tumor necrosis factor-alpha (TNF-α) significantly increased the ICAM-1 expression of fibroblasts in a dose-dependent manner. Treatment with IFN-β alone, however, did not up-regulate the ICAM-1 expression. Furthermore the attachment of peripheral blood mononuclear cells (PBMCs) to cytokine-treated fibroblasts was increased. This augmented attachment was partly inhibited by anti-ICAM-1 antibody. These results suggest that IFN-β and TNF-α may cooperatively modulate the attachment of PBMCs in the dermis.

Key words: IFN-β, TNF-α, ICAM-1; Fibroblast

1. Introduction

Intercellular adhesion molecule (ICAM)-1 is a cell surface glycoprotein expressed in many cell lines [1–3]. It belongs to a member of the immunoglobulin superfamily [4] and can function as an adhesive ligand for leukocyte function associated molecule (LFA)-1 [3,5]. The interaction between ICAM-1 and LFA-1 is thought to play an important role in immunologic and inflammatory responses such as leukocyte migration into dermis and epidermis [6–8]. In vitro, unstimulated human fibroblasts show low or undetectable ICAM-1 expression. It is known that the expression of ICAM-1 on fibroblasts can be up-regulated by cytokines such as tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin-1beta (IL-1β), and IL-4 [2,9–11]. However, as cytokines act simultaneously in the vivo situation, it is necessary to clarify how these cytokines form a network in modulating the expression of ICAM-1. Both IL-4 and IFN-γ stimulated the expression of ICAM-1 of fibroblasts and these cytokines display cooperative effects [11]. Thus it is important to investigate how these cytokines interact with one another in modulating the expression of ICAM-1.

Interferon was first characterized as an antiviral substance [12] and is believed to be an integral part of a cytokine network [13]. Interestingly, IFN-β is produced by human dermal fibroblasts [14] and its production is exponentially increased by a small amount of exogenously added IFN-β itself in vitro (unpublished data). However, the effects of IFNs on the expression of cell adhesion molecules have not been fully elucidated yet in fibroblasts. We show here the significant up-regulation of TNF-α-induced ICAM-1 expression by IFN-β on dermal fibroblasts.

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Abbreviations: IFN-β, interferon-beta; TNF-α, tumor necrosis factor-alpha; PBMCs, peripheral blood mononuclear cells; ICAM-1, intercellular adhesion molecule-1; LFA-1, leukocyte function associated molecule-1; IL-1, interleukin-; FITC, fluorescein isothiocyanate.

2. Materials and methods

2.1. Cell culture methods

Human dermal fibroblasts from surgically removed normal infant skins were isolated by explant outgrowth as previously described [15]. Cells were maintained in minimum essential medium (MEM) (Flow Laboratories) containing Earle's salt supplemented with 10% heat-inactivated fetal calf serum (FCS) (Flow Laboratories), penicillin (100 IU/ml) and streptomycin (100 μg/ml) (Gibco) in 5% CO_2 and 95% air at 37°C. Cells were detached from culture flasks by 0.05% trypsin/0.53 mM EDTA (Gibco, Grand Island, NY) and subcultured at a 1:3 split ratio. Five different strains were used between their 4th and 7th passages for the experiments.

2.2. Reagents

The following reagents were used for experiments. Human fibroblast IFN-β was a kind gift from Toray (Tokyo, Japan); human recombinant-TNF-α from Dainippon pharmaceutical Co. (Osaka, Japan); anti-ICAM-1 monoclonal antibody (MoAb) and FITC-conjugated anti-ICAM-1 MoAb (CD54) (mouse IgG1) were purchased from Immunotech (USA). Fluorescein isothiocyanate (FITC)-conjugated mouse immunoglobulin (IgG1) was from Becton Dicknson.

2.3. Flow cytometry

Fluorescence activated cell sorter (FACS) analysis was performed as previously described [15]. Human dermal fibroblasts were seeded in 25 cm^2 flasks (Falcon) at a density of $10^4/cm^2$. 24 h after seeding, attached cells were treated with TNF-α and IFN-β either alone or in combination at various concentrations for 48 h. Experiments were carried out using MEM containing low FCS (2%) to minimize the effect of serum on cytokines. Then, cells were detached from the dish by treatment with 1 mM EDTA. Cells were rinsed twice with phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+} containing 0.1% sodium azide and stained with FITC-conjugated purified anti-ICAM-1 MoAb (mouse IgG1) at 4°C for 30 min. As a negative control, isotype matched FITC-conjugated mouse immunoglobulin was used. After rinsing three times with PBS, cells were fixed with 1% paraformaldehyde. Samples were analyzed using a FACScan (Becton Dickinson). Data of FACS were expressed as log mean fluorescence.

2.4. Preparation of peripheral blood mononuclear cells (PBMCs)

Whole blood from normal healthy donors was collected into syringes containing heparin. Purified PBMCs were isolated by dextran sedimentation and Percoll (Pharmacia) gradient cenrtrifugation followed by hypotonic lysis of contaminating erythrocytes [16]. The purity of

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PBMCs was more than 90%. PBMCs were then suspended in MEM containing 1%FCS before assay.

2.5. Cell attachment assay

Semiconfluent cultures of fibroblasts in 96 well microplates (Falcon) were treated with 10 U/ml of TNF-α in the presence or absence of 1000 IU/ml of IFN-β for 48 h. PBMCs (3×10^5cells/100 μl of MEM) were then added to the cytokine treated fibroblasts for 30 min. Unattached cells were removed by rinsing three times with PBS. Then attached cells were stained with 0.25% (w/v) Rose bengal in PBS for 5 min [17]. After washing three times with PBS, dye incorporated into PBMCs was eluted with 100 μl of PBS : ethanol (1 : 1). The absorbance of eluted dye was read in a 2-wave length microplate photometer (Corona, Japan) at 570 nm. Results were presented after the subtraction of absorbance values of dye incooporated to fibroblasts. Cell number was directly proportional to the absorption of eluted dye [18]. The absorbance of dye incorporated into PBMCs adhering to TNF-α-untreated fibroblasts were set as controls. Data were expressed as % increase of control. For inhibition assay, cells treated by the same doses of TNF-α and IFN-β were incubated with 10 μg/ml of anti-ICAM-1 MoAb for 30 min. The final concentration of sodium azide was 0.001%, showing no inhibitory effect on cell activity. Then PBMCs were allowed to attach to fibroblasts for 30 min. The number of attached cells was calculated in the same way for cell attachment assay.

2.6. Statistical analysis

All experiments were performed at least three times. Results were shown as mean ± S.D. Statistical significance of data was analyzed using Student's *t*-test.

3. Results

3.1. Up-regulation of ICAM-1 by TNF-α

The effects of TNF-α on the expression of ICAM-1 of human cultured dermal fibroblasts were examined using flow cytometry. Non-treated human fibroblasts expressed very low levels of ICAM-1. When cells were treated with TNF-α for 48 h at concentrations of 1, 10, 100, and 1000 U/ml, the expression of ICAM-1 was significantly increased in a dose dependent manner (Fig. 1) as reported previously [2,9,11].

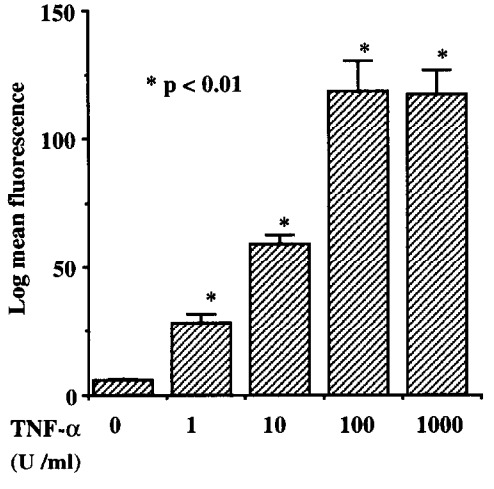

Fig. 1. Up-regulation of ICAM-1 by TNF-α. The expression of ICAM-1 of fibroblasts increased significantly in a dose dependent manner compared to that of controls when cells were treated with TNF-α for 48 h at concentrations of 1, 10, 100, and 1000 U/ml. Data of FACS are expressed as log mean fluorescence. All experiments were performed at least three times. Results are shown as mean ± S.D. $*P < 0.01$ compared to TNF-untreated cells. Statistical significance of data was analyzed using Student's *t*-test.

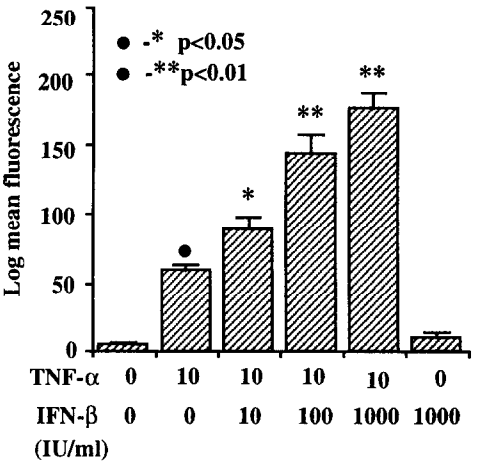

Fig. 2. Effect of IFN-β on TNF-α-induced ICAM-1 expression. When cells were treated with IFN-β at concentrations of 10, 100, and 1000 IU/ml in the presence of 10 U/ml of TNF-α, the expression of ICAM-1 could be up-regulated significantly above that seen by treatment of fibroblasts with TNF-α alone. Each up-regulation of ICAM-1 was statistically significant and dependent on the dose of IFN-β. The up-regulation of ICAM-1 was not observed by the treatment of 1000 IU/ml of IFN-β alone. Data of FACS are expressed as log mean fluorescence. All experiments were performed at least three times. Results are shown as mean ± S.D. ● $*P < 0.05$ and ● $**P<0.01$ compared to untreated cells. Statistical significance of data was analyzed using Student's *t*-test.

3.2. Effect of IFN-β on TNF-α-induced ICAM-1 expression

When cells were treated with IFN-β at concentrations of 10, 100, and 1000 IU/ml in the presence of 10 IU/ml of TNF-α for 48 h, the expression of ICAM-1 could be up-regulated significantly above that seen by treatment of fibroblasts with TNF-α alone (Fig. 2). However, the further up-regulation of ICAM-1 was not observed even if the concentrations of TNF-α were increased to 100 and 1000 U/ml in the presence of 1000 IU/ml of IFN-β (data not shown). The expression of ICAM-1 was not up-regulated by IFN-β alone at concentrations of 1, 10, 100, and 1000 IU/ml (data not shown).

3.3. Effect of IFN-β on the attachment of PBMCs to TNF-α-treated fibroblasts

As unstimulated PBMCs expressed LFA-1 [19], we investigated whether up-regulated ICAM-1 expression of fibroblasts by either IFN-β with TNF-α or TNF-α alone modulated the attachment of PBMCs. The number of PBMCs attached to fibroblasts in the presence of TNF-α (10 U/ml) increased by 12% compared to that of controls. More than twice the number of cells attached to fibroblasts were noted in the presence of IFN-β (1000 IU/ml) and TNF-α (10 U/ml).

These increases in the number of attached cells were statistically significant and dependent on the dose of IFN-β (data at doses of 10 and 100 IU/ml of IFN-β not shown). Furthermore it was noted that there was a tendency for attachment of PBMCs to either TNF-α alone or TNF-α with IFN-β-treated fibroblasts were inhibited by anti-ICAM-1 MoAb (Fig. 3).

4. Discussion

TNF-α has been shown to be a growth factor of human non-transformed fibroblasts [20,21]. We previously reported

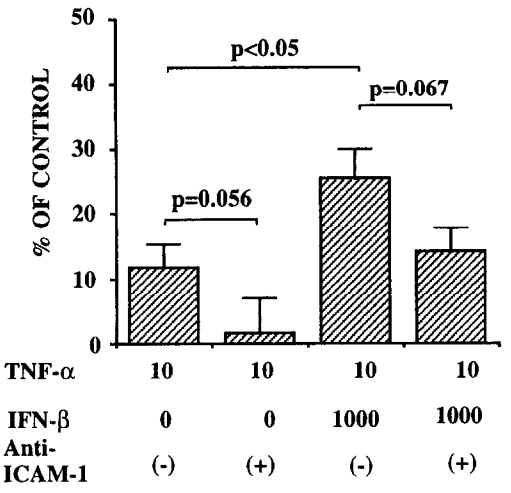

Fig. 3. Inhibitory effect of ICAM-1 MoAb on the attachment of PBMC to TNF-α and/or IFN-β fibroblasts. The number of PBMCs adhered to TNF-α (10 U/ml)-treated fibroblasts increased by 12% compared to that of controls. The treatment with 1000 IU/ml of IFN-β and 10 U/ml of TNF-α resulted in a more than twofold increase in the number of cells adhered to fibroblasts. This increase in the number of adhered cells was statistically significant ($P < 0.051$ compared to TNF-treated cells). Furthermore it was noted that there was a tendency that attachments of PBMCs to either TNF-α alone or TNF-α with IFN-β-treated fibroblasts were inhibited by anti-ICAM-1 MoAb. P values were, however, more than 0.05. Data were expressed as % increase of control.

that TNF-α preferentially induced the expression of α2β1 integrins of human skin fibroblasts in a dose- and time-dependent manner and increased the number of cells attached to type I collagen [15]. TNF-α was assumed to regulate the attachment and migration of fibroblasts in the processes of wound healing. A number of cytokines have been identified and some of their effects are known to regulate cell adhesion molecules. However, cytokines can have positive and negative biological effects and form a complex network in vivo [18,22]. For examples, in vitro, IL-4 decreases the IFN-γ-induced ICAM-1 expression of an endothelial cell line, whereas IL-4 alone has no effect on ICAM-1 expression. At the same time, IL4 could not up-regulate TNF-α-induced ICAM-1 expression [23]. Recently, it was reported that ICAM-1 is highly expressed on melanoma cells and its expression correlates with increased risk of metestasis [24,25]. In Japan, human IFN-β ($3–12 \times 10^6$ IU) has been widely accepted as an effective treatment and administered to the adjacent normal skin of the melanoma lesion [26]. We previously reported that IFN-β possessed anti-proliferative and anti-invasive effects on melanoma cells in vitro [27]. However, the skin immune and inflammatory responses exerted by locally administered IFN-β have not been fully explored. In the dermal tissues near the metastatic melanoma cells, mononuclear cell infiltration is rarely seen. This is appreciated to be due to the escape of melanoma cells from the immune surveillance system. After the local administration of IFN-β, however, infiltration of mononuclear cells are often observed near melanoma cells in the dermis. Moreover, the augmentation of ICAM-1 expression was not observed on melanoma cells but rather noted on dermal fibroblasts in the surrounding tissues in vivo (data not shown) [28]. The mechanism explaining the recruitment of infiltrating mononuclear cells has not been explained yet. Therefore we investigated the effects of IFN-β on the expression of ICAM-1 in cultured human dermal fibroblasts using flow cytometry. In our results, IFN-β significantly increased TNF-α- induced ICAM-1 expression in a dose-dependent manner, although IFN-β alone, even at concentration of 1000 IU/ml, did not up-regulate the expression of ICAM-1. It is known that significant concentrations of IFN-β are not detectable when it is locally administered. However, in lesional lymph nodes, high concentrations (200 to 1000 IU/g of wet tissue) of IFN-β were verified [29]. Thus the concentrations of IFN-β we emplyed in our experiments, ranging from 10 to 1000 IU/ml, was assumed to be compatible with the in vivo concentration. It is possible to assume that IFN-β regulates ICAM-1 expression in a cooperative way with TNF-α, resulting in the attachment of PBMCs into the site of inflammation and probably exerting an antitumoral effect. Further studies will be necessary to elucidate the function of PBMCs following the attachment to the fibroblasts through the interaction between ICAM-1 and LFA-1.

Investigations of a cytokine network in regulating ICAM-1 expression on dermal fibroblasts will lead to an understanding the mechanisms not only of various skin inflammatory diseases but also skin immune responses.

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