Difference in the Interferon-γ Induced ICAM-1 Expression Between Cultured Gingival and Epidermal Keratinocytes

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Abstract

An intercellular adhesion molecule 1 (ICAM-1) is an important adhesion molecule in the process of association between keratinocytes and activated lymphocytes. In order to characterize the expression of ICAM-1 in oral keratinocytes compared with that in epidermal keratinocytes, the effect of interferon gamma (IFN-γ) on the expression of ICAM-1 in cultured gingival and epidermal keratinocytes was investigated.

Materials and methods: Tissues of normal gingiva and skin were obtained during surgery. These tissues were cultured for about 1–2 weeks. After addition of IFN-γ to the medium, the expression of ICAM-1 was analyzed by using immunohistochemistry and flow cytometry in 5–48 hours.

Results: In immunohistochemistry, the expression of ICAM-1 in gingival keratinocytes was observed 24 hours after addition of 10^3 U/ml of IFN-γ, but was not observed at the concentration of 10^2 U/ml. While in epidermal keratinocytes, clear expression of ICAM-1 was detected at the concentration of 10^2 U/ml. In flow cytometry, the expression of ICAM-1 in gingival keratinocytes showed a low value compared with that in epidermal keratinocytes after 12, 24, and 48 hours at 10, 10^2 U/ml, and after 12 hours at 10^3 U/ml in statistical analysis. The results suggested that there are great differences in the sensitivity of IFN-γ induced ICAM-1 expression between oral and epidermal keratinocytes.

Key words: ICAM-1, IFN-γ, Keratinocytes, Oral mucous membrane

Acknowledgements

[Received Feb. 21, 2000, Accepted Mar. 27, 2000]

Introduction

Lichen planus shows an unique inflammatory cutaneous and mucous membrane reaction pattern of unknown etiology. Mucous membrane lesions are very common, occurring in 30–70% of cases with skin lesion: they are also not uncommonly found without evidence of skin lesion. Oral lichen planus may be located on the tongue, on the lip, and on the gingival margins, but the most common site is the buccal mucosa. Histologically, oral lichen planus is characterized by degeneration of the basal cells and the existence of band-like infiltrate predominantly containing lymphocytes. An intercellular adhesion molecule 1 (ICAM-1) is an important adhesion molecule in the process of association between keratinocytes and activated lymphocytes which is known to be T lymphocytes. However, the expression of ICAM-1 in oral lichen planus was less intense than that in cutaneous lichen planus, and not all investigators...
have been able to demonstrate ICAM-1 in keratinocytes of oral lichen planus. In order to characterize the expression of ICAM-1 in gingival keratinocytes compared with that in epidermal keratinocytes, the effect of interferon gamma (IFN-γ) on the expression of ICAM-1 in cultured gingival and epidermal keratinocytes was investigated by immunohistochemistry and flow cytometry.

**Materials and Methods**

1. **Immunohistochemistry**

Tissues of normal human gingiva and skin were obtained during surgical procedures with the agreement of patients and in accordance with the declaration of Helsinki. Gingival tissues were prepared from two male and three female patients (aged 17–29 yr) under extraction of tooth of impacted third molar. Epidermal tissues were prepared from one male and two female patients (aged 2–26 yr) during resection of benign tumor of back and face. These tissues were treated with dispase (500 protease unit/ml, Godo Shusei Co. Ltd, Japan) at 4°C overnight, and then epithelial layers were peeled off. After incubation in 0.025% trypsin/0.01% EDTA solution at 37°C for 10 minutes, single cell suspension was obtained. The cells (about 3.0 × 10⁴ cell/100 μl) were placed on coverslips in 35 mm dishes and fed with keratinocyte basal medium (KBM) (Clonetics, USA) supplemented 7.5mg/ml bovine pituitary extract, 0.1μg/ml epidermal growth factor, 0.5mg/ml hydrocortizone and 50 mg/ml gentamycinsulfate under 5% CO₂ at 37°C for 1–2 weeks. After addition of IFN-γ (Cellular Product Inc, USA) (10⁻¹⁰U/ml) to the medium, the expression of ICAM-1 was analyzed by immunohistochemistry after 5, 24, and 48 hours.

The cells were observed under fluorescent microscope (Nikon ECLIPS E800, Japan).

2. **Flow cytometry**

Tissues of normal human gingiva and skin were obtained during surgical procedures with the agreement of patients. Gingival tissues were prepared from two male and two female patients (aged 5 m–45yr) during extraction of tooth of impacted third molar and plasty of cleft lip. Epidermal tissues were prepared from three male and two female patients (aged 16–38yr and one case of cryopreservation neonatal skin) during resection of benign tumor of back, face, and femur. These tissues were treated and incubated in 60 mm dishes and the methods were the same as those used in immunohistochemistry.

After addition of IFN-γ (10⁻¹⁰U/ml) to the medium, the expression of ICAM-1 was analyzed by flow cytometry after 5, 12, 24, and 48 hours.

The cells were washed with PBS and treated with 0.025% trypsin/0.01% EDTA solution at room temperature (5–6 minutes). After the cells were released, the trypsin was neutralized in the dish with trypsin neutralizing solution (TNS) (Clonetics, USA). The detached cells were transferred to centrifuge tubes and centrifuged at 1500rpm for 5 minutes, 4°C. After the supernatant was aspirated, the cells were incubated with the first antibody (anti-ICAM-1 monoclonal antibody : Immunotech, France) for 1 hour on ice, followed by wash in PBS. The cells were then treated with the second antibody (FITC conjugated anti-mouse IgG antibody : Organon Teknika Corp, England) for 30 minutes and washed in PBS, fixed in formaldehyde solution (1% formaldehyde : PBS=1:1). The expression of ICAM-1 was analyzed using Cytron absolute (Orthoclinical Diagnostic, USA).

The results were statistically evaluated by Mann-Whitney U-test.

**Results**

1. **Immunohistochemistry**

Keratinocytes were almost confluent 1–2 weeks after initial culture and showed the characteristic pavement pattern by phase contrast microscopic observation (Fig. 1). In the gingival keratinocytes, the expression of ICAM-1 was observed diffuse and
somewhat homogeneous in the cytoplasm 5 hours after addition of $10^4$U/ml IFN-γ, and was observed as an intense linear pattern in the intercellular manner 24 hours after addition of $5 \times 10^3$U/ml IFN-γ (Fig. 2). It was not observed at all in IFN-γ untreated gingival keratinocytes (Fig. 3). The expression of ICAM-1 observed at the concentration of $10^2$ U/ml IFN-γ in gingival keratinocytes. However, at the same concentration of IFN-γ in epidermal keratinocytes, the expression of ICAM-1 was observed as a linear pattern in the intercellular manner (Fig. 4). It was not observed at all in IFN-γ untreated epidermal keratinocytes (Fig. 5). (Table 1, 2).

2. Flow cytometry

The positivity of ICAM-1 expression in gingival keratinocytes showed a low value compared with that in epidermal keratinocytes for all concentration of IFN-γ.

At the concentration of 10U/ml IFN-γ, the expression of ICAM-1 in epidermal keratinocytes was about twice as much as that in gingival keratinocytes. There are significant differences in the positivity of ICAM-1 expression after 12, 24, and 48 hours between gingival and epidermal keratinocytes ($p<0.05$) (Fig. 6).

At $10^2$U/ml IFN-γ, the expression of ICAM-1 in epidermal keratinocytes was more than that in gingival keratinocytes. The maximum level of ICAM-1 expression in epidermal keratinocytes was 93.1% at 12 hours, while that in gingival keratinocytes was 86.2% at 24 hours. There was a delay to the maximum level of ICAM-1 expression in gingival keratinocytes comparing with epidermal keratinocytes. There are significant differences in the positivity of ICAM-1 expression at 12, 24, and 48 hours ($p<0.05$).

The expression of ICAM-1 showed a wide range of SD in time until it reached the maximum level (Fig. 7).

At $10^3$U/ml IFN-γ, the expression of ICAM-1 in epidermal keratinocytes was more than that in gingival keratinocytes. The maximum level of ICAM-1 expression in epidermal keratinocytes was 93.7% at 12
Fig. 3 Immunohistochemical staining for ICAM-1 in IFN-γ untreated gingival keratinocytes. The expression of ICAM-1 was not observed at all.

Fig. 4 Immunohistochemical staining for ICAM-1 in IFN-γ untreated epidermal keratinocytes. The expression of ICAM-1 was observed as a linear pattern in the intercellular manner.

Fig. 5 Immunohistochemical staining for ICAM-1 in IFN-γ untreated epidermal keratinocytes. The expression of ICAM-1 was not observed at all.

Hours, while that in gingival keratinocytes was 87.4% at 24 hours. The time to the maximum level of ICAM-1 expression in gingival keratinocytes was also longer than that in epidermal keratinocytes. There are significant differences in the positivity of ICAM-1 expression at 12 hours (p<0.05).

The expression of ICAM-1 showed a wide range of SD in time until it reached the maximum level (Fig. 8).

Discussion
The expression of ICAM-1 has been reported on keratinocytes in the epidermal immunoinflammatory disease such as lichen planus and was well correlated with T lymphocytes infiltration in the subepithelia8,9). ICAM-1 is considered to be an important adhesion molecule in the process of association between keratinocytes and activated T lymphocytes as a
Fig. 6 The percentage of ICAM-1 positive cells on gingival and epidermal keratinocytes induced by IFN-γ (10U/ml).
After addition of 10U/ml IFN-γ to the cultured gingival and epidermal keratinocytes, the expression of ICAM-1 was analyzed by using flow cytometry in 5, 12, 24, and 48 hrs. The expression of ICAM-1 in epidermal keratinocytes was about twice as much as that in gingival keratinocytes. There are significant differences in the positivity after 12, 24, and 48 hrs (p<0.05). Each point represents the mean±SD.

Fig. 7 The percentage of ICAM-1 positive cells on gingival and epidermal keratinocytes induced by IFN-γ (10²U/ml).
After addition of 10²U/ml IFN-γ to the cultured gingival and epidermal keratinocytes, the expression of ICAM-1 was analyzed by using flow cytometry in 5, 12, 24, and 48 hrs. The positivity of ICAM-1 expression in epidermal keratinocytes achieved maximum level at 12 hrs, while maximum level of gingival keratinocytes at 24 hrs. There are significant differences in the positivity of ICAM-1 expression after 12 hrs (p<0.05). Each point represents the mean±SD.

Fig. 8 The percentage of ICAM-1 positive cells on gingival and epidermal keratinocytes induced by IFN-γ (10³U/ml).
After addition of 10³U/ml IFN-γ to the cultured gingival and epidermal keratinocytes, the expression of ICAM-1 was analyzed by using flow cytometry in 5, 12, 24, and 48 hrs. The positivity of ICAM-1 expression in epidermal keratinocytes achieved maximum level at 12 hrs, while maximum level of gingival keratinocytes at 24 hrs. There are significant differences in the positivity of ICAM-1 expression after 12 hrs (p<0.05). Each point represents the mean±SD.
keratinocytes may not be essential adhesion molecules for T lymphocyte infiltration in oral lichen planus, and oral keratinocytes have different sensitivity to cytokine of epidermal keratinocytes.

Since oral mucosa is under a severe environment which is always irritated by foods, bacteria, virus, dental materials, drugs, and so on, immunoresponsibility in oral mucosa can be maintained weaker than that in skin and other mucous membranes such as nasal mucosa and branchial mucosa.

Although detailed mechanisms of the differences in sensitivity to IFN-γ between oral and epidermal keratinocytes are unclear, the results indicated one of the biological evidences which explain cellular mechanism of the immune system in the oral cavity. More detailed experiments must be done to confirm this.

References

歯肉と皮膚上皮細胞における IFN-γ が誘導する ICAM-1 の発現量の比較

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ICAM-1 は、上皮細胞と活性化 T 細胞の接着過程において重要な役割を果たす接着分子の一つである。今回われわれは、口腔粘膜と表皮上皮細胞における ICAM-1 の発現状態の違いを解析する目的で、ヒト歯肉ならびに皮膚由来培養上皮細胞を用い、ICAM-1 の発現について IFN-γ の影響を観察した。

材料と方法：検体となる歯肉ならびに皮膚は手術の際に採取し、1〜2 週間培養後、培地に IFN-γ を加え 5〜48 時間後に免疫組織化学的ならびにフローサイトメトリーにて ICAM-1 の発現を観察した。

結果：免疫組織化学的観察において、歯肉では IFN-γ 10^3 U/ml 添加 24 時間後で ICAM-1 の発現を認めたが、10^2 U/ml では発現は認めなかった。一方、皮膚では、10^3 U/ml で発現を認めた。フローサイトメトリーでの解析では、10, 10^2 U/ml 添加 12, 24, 48 時間後と 10^3 U/ml 添加 12 時間後において、皮膚に比べ歯肉は ICAM-1 の発現量が統計学的に有意に低い結果であった。

われわれの結果は、口腔粘膜と皮膚上皮細胞では ICAM-1 発現における IFN-γ の感受性に違いがあることを示唆した。

キーワード：ICAM-1, IFN-γ, 上皮細胞, 口腔粘膜