Effect of Interleukin-1β on the Expression of Tight Junction Proteins in the Culture of HaCaT Keratinocytes

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 3, pp. 251-254, March, 2010
Original article submitted August 26, 2009

We studied the effect of IL-1β on the expression of tight junction proteins (occludin and claudins) in cultured HaCaT keratinocytes and changes of transepithelial resistance. Addition of IL-1β had little effect on transepithelial resistance, increased the expression of claudin-1, and did not modify the expression of occludin. In other tissues, IL-1β also increases claudin-1 expression, but significantly decreases occludin expression. These changes are accompanied by the reduction of transepithelial resistance. The IL-1β-induced increase in the expression of claudin-1 in cultured HaCaT keratinocytes simulates the appearance of claudin-1 at the early stage of skin wound healing. It is accompanied by an increase in IL-1β concentration in the wound fluid.

Key Words: *HaCaT; claudin; occludin; interleukin-1β*

The superfamily of IL-1 includes more than 10 homologous cytokines. The major cytokines are IL-1β and IL-1α, IL-1 receptor antagonist, and IL-18. IL-1β is produced by keratinocytes and Langerhans cells of the skin and plays a role in migration of these cells [5]. IL-1β induces production of other proinflammatory cytokines, including IL-8. Differentiation of keratinocytes is accompanied by variations in IL-1β expression [8]. During skin wound healing, IL-1β induces leukocyte migration to the wound area and stimulates migration and proliferation of epithelial cells and connective tissue cells [10]. The expression of IL-1β mRNA and the concentration of IL-1β in the wound fluid increase at the early stage of wound process [6]. The use of IL-1β as a medicinal product improves skin wound healing [13]. IL-1β in high concentrations modulates the expression of tight junction proteins (ETJP) in the culture of HaCaT keratinocytes [14]. However, *in vitro* effect of long-term treatment with IL-1β in low doses on ETJP remains unknown.

ETJP in stratified squamous epithelium maintains the epidermal barrier in the skin [7]. The synthesis and distribution of tight junction proteins during various periods after removal of the epidermal barrier were studied on the experimental model of human wound healing. Tight junction proteins (claudin-1, occludin, and ZO-1) were shown to appear at the early stage of wound healing. They were found in primary cells of the epithelial cell group before reconstruction of the horny layer [4].

In vivo models do not allow us to evaluate the direct effect of IL-1β on ETJP during tissue regeneration. Therefore, culture of HaCaT keratinocytes was used as an experimental model. This work was designed to study ETJP in the culture of HaCaT keratinocytes after treatment with IL-1β.

MATERIALS AND METHODS

ETJP in culture was studied by Western blotting followed by densitometry. The culture of epithelial cells from the large intestine (HT-29/B6) expressing occludin, claudin-1, claudin-2, claudin-3, and claudin-5 was used as a positive control in Western blotting. Occludin and claudin-1 were analyzed during densitometry to evaluate the effect of IL-1β on ETJP. Occludin is a marker of tight junctions. Claudin-1 is an important

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0007-4888/10/1493-0280

structural protein determining functional activity of tight junctions in the skin [7]. The rate of formation of tight junctions was estimated by measuring the transepithelial resistance (TER). IFN-γ served as the positive control. IFN-γ is an important modulator of innate and acquired immunity. This anti-inflammatory agent triggers macrophage activity. Published data show that IFN-γ increases significantly ETJP in the culture of HaCaT keratinocytes [9].

HaCaT keratinocytes were cultured in RPMI medium, which consisted of 10% bovine serum (Biochrom), 600 mM L-glutamine, 500 U penicillin, and 500 μg/ml streptomycin (Seromed/Biochrom). Culturing was performed in culture plates (20×10 mm; Falcon, Becton Dickinson) at 37°C, 5% CO_2, and 100% humidity.

Human recombinant IL-1β in a concentration of 10 ng/ml medium was added to cells of the treatment group. IFN-γ in a concentration of 10 ng/ml medium was added to positive control cells for the measurement of TER. The cells were scraped off from the surface of a culture plate on day 7 after attaining the confluence. The cell suspension was placed in an Eppendorf tube and mixed by 10-fold passing through an insulin syringe. The suspension was centrifuged at 200*g* and 4°C for 5 min to separate the fractions of membrane and cytoplasm proteins. The soluble fraction containing membrane proteins was centrifuged at 43,000*g* and 4°C for 30 min. Cold Tris-buffer (100 μl) was added to the pellet. It was pumped several times to achieve complete solution of the pellet.

The plate was filled routinely to evaluate the total content of membrane proteins in the solution. Bidistilled water, lysing buffer, and BSA solutions (0.2, 0.8, and 1.2 μg/μl; Pierce, Rockford) were used as the control. The concentration of membrane proteins in test samples was measured relative to the control. The wells were filled with test samples. The plate was maintained in a thermostat at 37°C for 30 min and then cooled to room temperature. The concentration of membrane proteins in the test sample was estimated in a spectrophotometer (Tecan Spectra) at 562 nm. The volume of the solution of membrane proteins for electrophoresis was calculated from the estimated values.

Electrophoresis of membrane protein aliquots was performed in PAAG with sodium dodecyl sulfate. After electrophoresis, these proteins were transferred from the gel to a hydrophobic membrane (electrical transport at a constant voltage of 100 V for 60 min). The membranes were washed in phosphate buffer with detergent. They were blocked in the same solution with BSA (final concentration 5%) for 120 min. The membranes were incubated with primary antibodies (Zymed Laboratories) to the tight junction marker occludin (1:2000); claudin-2 (1:1000); claudin-1, claudin-7, claudin-8, claudin-10, claudin-11, claudin-12, claudin-14, claudin-15, claudin-18, and claudin-23 (1:2000); and claudin-3, claudin-4, and claudin-5 (1:5000). Further incubation was performed with horseradish peroxidase-conjugated polyclonal secondary antibodies (Roche Diagnostics). The membrane was developed in a chemiluminescent solution (Lumilight; Roche Diagnostics) over 5 min for protein visualization. Detection was conducted in a LAS 1000 image analyzer (Fujifilm). The results of Western blotting were analyzed using AIDA software. Densitometry was performed.

TER of the HaCaT culture was measured daily on a Millicell-ERS epithelial voltmeter (Millipore) for 7 days. One electrode was placed at the apical surface of cells. The other electrode was localized at the basal surface.

The results were analyzed by Mann–Whitney test. The differences were significant at $p<0.05$.

RESULTS

Western blotting analysis was performed with 15 tight junction proteins from the control group of cultured HaCaT keratinocytes. Only five proteins were revealed (occludin, claudin-1, claudin-3, claudin-12, and claudin-14; Fig. 1). Occludin was identified as the protein with a molecular weight of 60 kDa. The molecular weight of claudins was 23 kDa. The signal of claudin-12 and claudin-14 was not detected in the culture of HT-29/B6. The culture does not express these claudins under normal conditions. The results of Western blotting are consistent with those of a Northern blotting analysis demonstrating the presence of mRNA for occludin, claudin-1, and claudin-3 in culture of HaCaT keratinocytes [12]. We showed for the first time that keratinocytes contain claudin-14. The expression spectrum of claudins differs from the composition of tight junction proteins in mammalian skin, which expresses occludin, claudin-1, claudin-4, claudin-7, claudin-8, claudin-11, claudin-12, claudin-17, and claudin-18 [3,4]. However, the structure of tight junctions does not include pore-forming proteins (*e.g.*, claudin-2, claudin-6, and claudin-16). Our results are consistent with published data that tight junctions serve as an impermeable barrier of the skin.

Densitometry of the signals from claudin-1 and occludin showed that addition of IL-1β to cultured cells significantly increased claudin-1 signal (by 4 times compared to the control; Fig. 2). The signal of occludin increased insignificantly under these conditions. Therefore, IL-1β has a direct effect on the expression of claudin-1. Our results are consistent with published data that IL-1β modifies ETJP in cultures of other cells: IL-1β stimulates claudin-1 production in culture of Caco-2 intestinal epithelial cells [2]. Addi-

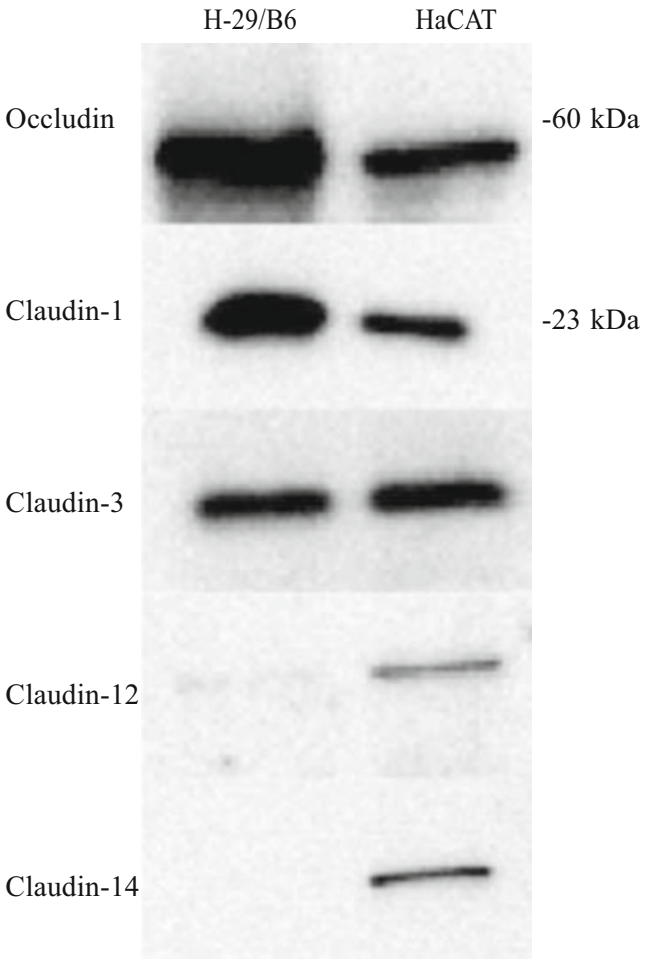

Fig. 1. Expression of tight junction proteins in cultures of HaCaT cells and H-29/B6 cells.

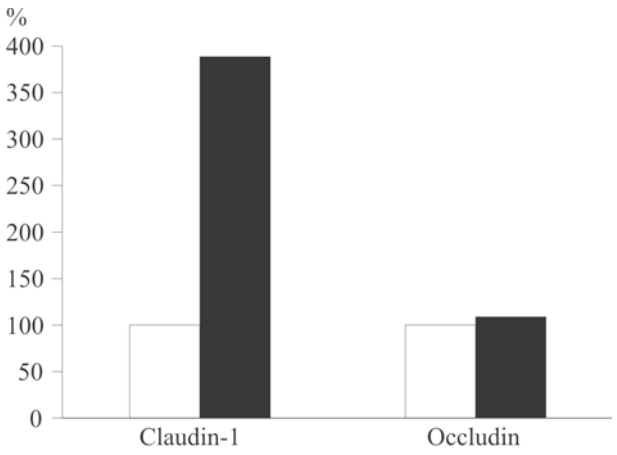

Fig. 2. Signals of occludin and claudin-1 after addition of IL-1β to the culture of HaCaT cells. Light bars, control; dark bars, IL-1β.

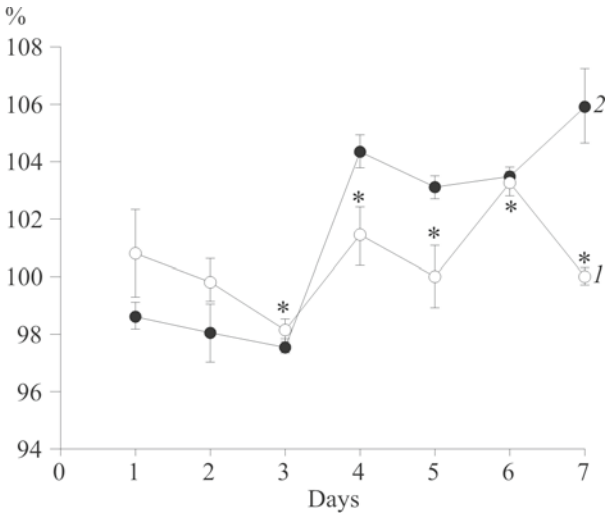

Fig. 3. Effect of IL-1β (*1*) and IFN-γ (*2*) on TER of cultured HaCaT cells. TER in the control is taken as 100%. **p*<0.05 compared to *2*.

tion of IL-1β to cultured cells of the retinal pigment epithelium was followed by an increase in claudin-1 expression. These data were obtained in real-time PCR, immunohistochemical study and Western blotting analysis [1].

The data obtained in our experiments were compared with the results of studying the effect of IL-1β on keratinocytes. It should be emphasized that short-term *in vitro* exposure of the HaCaT cell culture to IL-1β in high concentrations induced opposite changes in ETJP. Addition of IL-1β (1 μg/ml) to the confluent culture of HaCaT cells (48 h) significantly decreased the expression of claudin-1 and claudin-3 by cultured keratinocytes [14]. By contrast, IFN-γ and TNF-α did not modify the expression of claudin-1 and claudin-3 at the protein level (results of Western blotting). Psoriasis is characterized by increased concentration of IL-1β. The disease serves as an experimental model of this situation [15]. Similarly to the *in vitro* effect of IL-1β in high doses on ETJP, psoriasis is characterized by reduced expression of claudin-1 [14]. It can be hypothesized that IL-1β produces a dose-dependent effect on claudin-1 expression in the culture of HaCaT keratinocytes.

IFN-γ significantly increased TER, while IL-1β was ineffective in this respect (Fig. 3).

The IL-1β-induced increase in claudin-1 expression in the culture of HaCaT keratinocytes simulates the appearance of claudin-1 at the early stage of skin wound healing. These changes are accompanied by an increase in IL-1β concentration in the wound fluid. It was hypothesized that skin injury is followed by induction of ETJP. They prevent excessive loss of fluid through the wound surface and protect the body until the formation of the horny layer [11]. The results of our study provide support for this hypothesis.

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