論文の内容の要旨

論文題目:

High Calcium, ATP and Poly(I:C) Augment the Immune Response to β-glucan in Normal Human Epidermal Keratinocytes
（高濃度カルシウム、ATP，Poly(I:C)はヒトケラチノサイトのβ-グルカンに対する免疫学的反応性を増強する）

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Contact with fungal and bacterial pathogens initiates a series of host responses, beginning with innate immunity. β-glucans are pathogen associated molecular patterns of fungi such as *Candida albicans*. Dectin-1 has been reported as the major receptor for β-glucans in studies done mostly on macrophages and dendritic cells, but not on keratinocytes. Here, we studied the effects of β-glucan on normal human epidermal keratinocytes (NHEK) from neonatal foreskin. We were also interested in investigating the effect of β-glucan on NHEK especially during conditions of skin damage and infection, which would allow deeper penetration of the fungi through impaired skin. We co-stimulated the cells with high calcium to induce keratinocyte differentiation, mimicking the uppermost layer of the epidermis; ATP, a danger signal released by UVB-exposed and damaged keratinocytes; and pathogen-associated compounds such as the TLR3 ligand, poly(I:C), found in viral infections; and the TLR4 ligand, LPS, found in

bacterial infections. We also aimed to determine the possible role of dectin-1 in the process.

Subconfluent NHEK were stimulated with β-glucan 20 μg/ml or an equal amount of 0.3M NaOH, used to dissolve the β-glucan preparation. NHEK were pre-treated with either 0.05mM or 1.3 mM calcium for 24 hours, or ATP 250 uM, poly(I:C) 25 μg/ml, or LPS from *E. coli* or *S. minnesota* 10 ng/ml for 1 hour prior to β-glucan stimulation. Supernatants were collected at 12, 24 and 48 hours after stimulation with β-glucan. IL-8/CXC chemokine ligand 8, IL-1α, IL-6, IL-10, IL-12p40, IL-23, tumor necrosis factor-α, thymus and activation-regulated chemokine (TARC)/CC chemokine ligand 17, macrophage inflammatory protein (MIP)-3α/CC chemokine ligand 20 concentrations in the supernatants were measured by ELISA.

Cells were harvested and assessed for dectin-1 expression by reverse-transcription-PCR and flow cytometry analysis. Deectin-2 expression was also investigated using RT-PCR. Cell lysates of NHEK stimulated with β-glucan at 0, 5, 10, 15, 20, 30 and 60 minutes were used in immunoblotting to determine ERK, p38 MAPK, Akt or IKK α/β phosphorylation. Likewise, lysates of NHEK treated with high calcium, ATP or poly(I:C) alone were compared to lysates of NHEK pre-treated with these compounds and then stimulated with β-glucan. To assess ERK and p38 MAPK inhibition respectively, NHEK were pre-treated with the ERK inhibitor PD98059 or p38 MAPK inhibitor SB203580 prior to β-glucan stimulation. Supernatants were harvested after 24 hours to assess for IL-8 production, while cell lysates were prepared for immunoblotting.

NHEK responded to β-glucan, which induced significantly elevated levels of IL-8, and IL-6 compared to controls at 12, 24 and 48 hours. NaOH, reagent used to dissolve β-

glucan, did not affect cytokine or chemokine secretion. No significant elevations were found in the other cytokines or chemokines tested.

β-glucan also induced higher levels of IL-8 secretion from well-differentiated NHEK compared to undifferentiated ones. No significant elevations in IL-1α and IL-6 were seen in well-differentiated NHEK treated with β-glucan. ATP upregulated IL-8 and IL-6 production in β-glucan-stimulated NHEK compared to β-glucan or ATP stimulation alone. IL-1α was not upregulated. The transient elevation of IL-1α noted in β-glucan-stimulated NHEK at 24 hours by ELISA was not found at the transcription level. However, poly(I:C) significantly upregulated IL-1α secretion in β-glucan-stimulated cells versus cells stimulated with β-glucan or poly(I:C) alone. No upregulation was noted for IL-8 or IL-6. No response to LPS was seen in NHEK, probably due to a very low expression of TLR4 in these cells.

We also found that NHEK expressed dectin-1 in both the mRNA and protein level by RT-PCR and flow cytometry analysis. Its cell surface expression was downregulated by β-glucan stimulation with flow cytometry analysis, signifying internalization of β-glucan by the cells. Dectin-2 was not expressed in NHEK.

The induction of ERK and p38 MAPK signaling pathways by fungal particles via the dectin-1 receptor had been studied using dendritic cells and macrophages. Thus we studied the induction of these two pathways in β-glucan-stimulated NHEK. Our results showed that β-glucan specifically induced the responses in NHEK via the ERK and p38 MAPK pathways. Although minimal activation of ERK and p38 MAPK was present when NHEK were treated with high calcium, ATP or poly(I:C) alone, addition of β-glucan significantly augmented ERK and p38 MAPK activation. Moreover, the addition

of the ERK inhibitor PD98059 and the p38 MAPK inhibitor SB203580 effectively suppressed the IL-8 secretion in β-glucan-stimulated NHEK. Akt and IKKα/β phosphorylation was negative in NHEK stimulated with β-glucan.

In conclusion, high calcium, ATP and pathogen-derived components like poly(I:C) can augment inflammation in β-glucan-stimulated NHEK, leading to a rapid and effective host defense against cutaneous fungal infections. Dectin-1 expressed on NHEK may play an essential role in the responses to β-glucans by these cells, although further studies are needed to fully evaluate its role.