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Focal adhesion kinase mediates human leukocyte histocompatibility antigen class II-induced signaling in gingival fibroblasts

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Background and Objective: The role of human leukocyte antigen class II molecules on nonantigen-presenting cells has been a matter of controversy. We previously reported that human leukocyte antigen class II molecules on human gingival fibroblasts do not present antigens, but transduce signals into the cells by making a complex with antigenic peptide T-cell receptor or by stimulating cell surface human leukocyte antigen-DR molecules with human leukocyte antigen-DR antibody (L243), which mimics the formation of the human leukocyte antigen class II–antigenic peptide T-cell receptor complex, resulting in the expression of several cytokines. The aim of this study was to detect human leukocyte antigen class II-associated molecules mediating human leukocyte antigen class II-induced signals into the cells.

Material and Methods: Antibody-based protein-microarray analysis was performed to detect activated signaling molecules in gingival fibroblasts stimulated via human leukocyte antigen class II molecules. Then, we examined if these molecules structurally associate with human leukocyte antigen class II and actually transduce signals into the cells.

Results: Stimulation of human leukocyte antigen class II on gingival fibroblasts by L243 resulted in enhanced phosphorylation of focal adhesion kinase. Focal adhesion kinase was co-immunoprecipitated with human leukocyte antigen-DR by L243. Stimulation of gingival fibroblasts with L243 induced phosphorylation of focal adhesion kinase. Luteolin, a putative focal adhesion kinase inhibitor, suppressed phosphorylation of focal adhesion kinase and dose dependently inhibited human leukocyte antigen class II-induced cytokine production.

Conclusion: Focal adhesion kinase is structurally associated with human leukocyte antigen-DR and mediates human leukocyte antigen class II-induced signals in gingival fibroblasts.

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The physiological role of human leukocyte antigen class II molecules on nonantigen-presenting cells, such as fibroblasts, has not yet been fully elucidated, although these cells are known to express human leukocyte antigen class II molecules on their cell surface, particularly highly upon stimulation with interferon-γ. For

example, gingival fibroblasts in inflamed periodontal tissues have been suggested to express human leukocyte antigen class II molecules on their cell surface (1). We previously reported that the ligation of human leukocyte antigen-DR molecules on cultured human gingival fibroblasts with human leukocyte antigen-DR monoclonal antibodies (L243), or with antigenic peptide/T-cell-receptor complex, resulted in the secretion of several cytokine/chemokines, such as regulated upon activation, normal T-cell expressed and secreted (RANTES), monocyte chemoattractant protein 1 and interleukin-6, and suggested that cytokines thus secreted could further enhance the deterioration of the clinical course of inflammatory periodontal diseases (2). Additionally, we recently reported that the ligation of human leukocyte antigen-DR molecules with antibody to DR on interferon-γ-stimulated fibroblasts resulted in activation of the intracellular signaling molecule, c-jun N-terminal kinase, a mitogen-activated protein kinase (MAPK), and c-jun N-terminal kinase activity is at least involved in the RANTES production in such cells, as specific inhibitor for c-jun N-terminal kinase inhibited human leukocyte antigen class II-induced RANTES expression in these cells (3). However, to date, it is still unclear as to what molecules structurally associate with human leukocyte antigen-DR molecules in nonantigen-presenting cells, and, if any, whether such molecules transduce signals into the cells. Because it is known that the intracellular domain of human leukocyte antigen class II molecules is quite short in length, and that this domain even lacks the amino acid residues potentially receiving phosphorylation which transduce signals into the cells, some other molecules that structurally associate with human leukocyte antigen class II molecules are actually suggested to mediate human leukocyte antigen class II-induced signals (4). Therefore, identification and characterization of human leukocyte antigen class II-associated molecules, which potentially transduce human leukocyte antigen class II-induced signals into nonantigen-presenting cells, would greatly enhance our understanding of the overall physiological role of human leukocyte antigen class II molecules expressed on nonantigen-presenting cells. In particular, it is important to identify such molecules in specific cell types, such as gingival fibroblasts, which are easily exposed to chronic inflammatory conditions, because human leukocyte antigen class II molecules expressed on these cells under inflammatory conditions may greatly alter the clinical course of inflammatory periodontal diseases.

Therefore, in this study, in an attempt to identify associate molecules with human leukocyte antigen-DR and putative molecules mediating human leukocyte antigen-DR-induced signals in gingival fibroblasts, we first utilized an antibody-based protein microarray technique. We then attempted to establish if such molecules structurally associate with human leukocyte antigen-DR molecules and actively mediate human leukocyte antigen class II-induced signaling into the cells.

Material and methods

Reagents

Mouse monoclonal antibodies raised against human leukocyte antigen-DR (L243; Leinco Technologies Inc., Ballwin, MO, USA) were used for ligation with human leukocyte antigen-DR molecules. Isotype-matched control mouse IgG2a was obtained from Pharmingen (San Diego, CA, USA). Interferon-γ was obtained from Genzyme (Cambridge, MA, USA). As putative inhibitors for focal adhesion kinase, luteolin and quercetin were used. Luteolin was purchased from Extrasynthese (Genay, France), and quercetin was from Nacalai Tesque (Kyoto, Japan).

Cells and cell culture

Human gingival fibroblasts were isolated from the gingival tissues of healthy volunteers during the extraction of an impacted third molar. Gingival tissues were plated in a 35-mm cell culture dish precoated with type I collagen and fibronectin. Outgrown cells from tissue explants were expanded and maintained as described previously (2). Briefly, the cells were cultured in a medium composed of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 10 mg/mL of gentamicin, 0.1 mM nonessential amino acids, vitamins and 2 mM L-glutamine. When the cells reached confluence, they were passaged with a split ratio of 1 : 4. The cells were used between the fifth and seventh passages for all experiments.

Antibody microarray analyses

To detect putative signaling molecules in fibroblasts stimulated with L243, activated molecules were detected by an antibody-based protein-microarray technique (Panorama TM Ab Microarray; Sigma, St Louis, MO, USA). The cells were first cultured until reaching subconfluency, and the medium was then changed to serum-free medium and the cells cultured for 24 h for serum starvation, followed by 48 h of culture, with or without 500 U/ml of interferon-γ in serum-free medium. Then, the cells were stimulated either with L243 (1 μg/ml) or with control IgG. At the indicated time point, the cells were lysed with cell lysis buffer included in the microarray kit and the soluble fractions were obtained. The cellular proteins were labeled with either of the fluorescent dyes, Cy3 or Cy5, and the labeled proteins were subjected to microarray analyses according to the manufacturer's instructions. The fluorescence intensity of each reaction was measured by using a GenePix 4000B Microarray scanner (Amersham Biosciences, Bucks., UK), and the data were quantified by using ARRAY VISION software (Amersham Biosciences).

Immunoprecipitation

To isolate putative associate molecules with human leukocyte antigen-DR, interferon-γ-treated fibroblasts for 48 h were first lysed with 1% CHAPS buffer containing 150 mM NaCl, 20 mM Tris-HCl, 1 mM NaF and 5 mM EDTA, and the soluble fraction was obtained by centrifugation. Then, the lysates were incubated with L243, and

the L243–protein complex was immunoprecipitated with Protein A Magnetic Beads (New England Biolabs, MA, USA), according to the manufacturer's instructions. The complex was then subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis. In some experiments, the cells were first stimulated either with L243 or with control IgG for the indicated time period and then lysed, followed by immunoprecipitation with Protein A magnetic beads.

Western immunoblotting

Detection of tyrosine-phosphorylated focal adhesion kinase and of focal adhesion kinase proteins were performed by western immunoblotting. Briefly, protein samples obtained by immunoprecipitation, as described above, were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and blotted onto a poly(vinylidene difluoride) membrane. To detect focal adhesion kinase, rabbit antihuman focal adhesion kinase polyclonal antibody (Sigma, St Louis, MO, USA) was used as the primary antibody, whereas to detect phospho-focal adhesion kinase, rabbit antiphospho-focal adhesion kinase polyclonal antibody (pY397: Sigma) was used as the primary antibody. Horseradish peroxidase-conjugated antirabbit IgG (Amersham Biosciences) was used as the secondary antibody. Immunoreactive proteins were detected by the enhanced chemiluminescence method (Amersham Biosciences).

In some experiments, the cells were cultured with or without the concentration indicated of luteolin or quercetin (i.e. putative focal adhesion kinase inhibitors) for 48 h, and the cells were lysed with cell lysis buffer (1% CHAPS, 2 mM Na_3VO_4, 1 mM NaF, 5 mM EDTA and proteinase inhibitor mixture; Roche Diagnostics GmbH, Mannheim, Germany) in 20 mM Tris-150 mM NaCl buffer, pH 7.6) at each indicated time point. Protein concentration was measured by protein assay (Bio-Rad, Hercules, CA, USA). Ten micrograms of protein sample was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and blotted onto the membrane. Phospho-focal adhesion kinase and focal adhesion kinase were detected as described. To determine the effects of luteolin on the phosphorylation of extracellular signal-regulated kinase (ERK), p38 and c-jun N-terminal kinase MAPKs, and on c-jun, some samples were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis followed by western immunoblotting with rabbit antiphospho-ERK, ERK, phospho-p38, p38, phospho-c-jun N-terminal kinase polyclonal antibody, c-jun N-terminal kinase polyclonal antibody, phospho c-jun polyclonal antibody and c-jun polyclonal antibody (all from Cell Signaling, Beverly, MA, USA), as luteolin has been suggested to influence the phosphorylation of these proteins in other cell types. In these experiments, the membranes were first probed with the antibodies against each native protein and then reprobed with the antibodies recognizing the phosphorylated form of each protein after stripping bound antibodies with stripping buffer (2% sodium dodecyl sulfate and 100 mM mercaptoethanol in 62.5 mM Tris-HCl buffer, pH 6.7).

Cytokine assay

The concentration of RANTES, monocyte chemoattractant protein 1 and interleukin-6 in the cell culture supernatants stimulated with human leukocyte antigen-DR molecules, with or without pre-incubation of the cells with the indicated concentration of luteolin for 48 h, was measured by using a commercial immunoassay kit (human RANTES, monocyte chemoattractant protein 1 and interleukin-6 enzyme-linked immunosorbent assay kit; Endogen Inc., Woburn, MA, USA) following 16 h of cell culture.

Statistical analyses

Statistical analyses comparing the cytokine productivity between the cells stimulated with L243 in the presence or absence of luteolin was performed by using the Student's *t*-test.

Results

Ligation of human leukocyte antigen-DR molecules on fibroblasts with L243 results in the activation of several signaling molecules

Ligation of human leukocyte antigen-DR molecules with L243 resulted in the enhanced phosphorylation of several molecules, including focal adhesion kinase, Raf and c-jun N-terminal kinase (Fig. 1). Approximately twofold higher phosphorylation was observed in each kinase compared with the cells

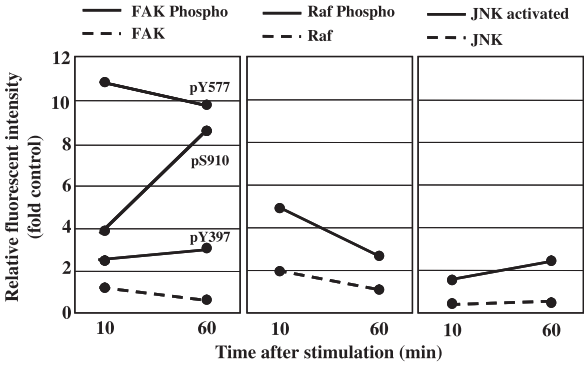

Fig. 1. Ligation of human leukocyte antigen-DR molecule on fibroblasts with L243 induces enhanced phosphorylation of focal adhesion kinase, c-jun N-terminal kinase and Raf-1. The cells were stimulated with either L243 or control IgG. The cell lysates were obtained at indicated time point, labeled with fluorescent dye and subjected to protein microarray analyses, as described in the Material and methods. Data are expressed as fold increase of fluorescence intensity of test samples against that of control samples obtained from the cells stimulated with isotype-matched control IgG. FAK, focal adhesion kinase; JNK, c-jun N-terminal kinase.

stimulated with control IgG. As for focal adhesion kinase, all three antibodies recognizing distinct phosphorylation sites (pY397, pS910 and pY577) reacted strongly with the proteins obtained from L243-stimulated cells, suggesting that focal adhesion kinase is highly activated by L243 stimulation.

Human leukocyte antigen-DR molecules on fibroblasts directly associate with focal adhesion kinase

As focal adhesion kinase usually associates with the intracellular domain of integrins, we next attempted to determine whether human leukocyte antigen-DR molecules on fibroblasts directly associate with focal adhesion kinase. Cellular proteins, co-immunoprecipitated with either L243 or control IgG, were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and focal adhesion kinase was detected by western immunoblotting. As shown in Fig. 2, focal adhesion kinase was clearly observed in a fraction immunoprecipitated with L243 obtained from interferon-γ-stimulated cells. A weak band was also observed in a fraction immunoprecipitated with L243 obtained from the cells without prior interferon-γ stimulation. In contrast, no corresponding band was detected in a fraction immunoprecipitated with control IgG. Thus, this result indicates that human leukocyte antigen-DR molecules on fibroblasts directly associate with focal adhesion kinase.

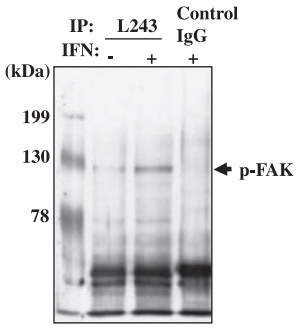

Fig. 2. Focal adhesion kinase directly associates with human leukocyte antigen-DR molecules on fibroblasts. Interferon-γ-treated or -untreated fibroblasts were lysed, and the cell lysates were immunoprecipitated with L243, followed by western immunoblotting with phospho focal adhesion kinase antibody (pY397). As a control, interferon-γ-treated cells were similarly lysed, and the lysates were immunoprecipitated with control IgG. Focal adhesion kinase was highly detected in a fraction immunoprecipitated with L243 obtained from the cells stimulated with interferon-γ. Weak reactivity was also observed in a fraction immunoprecipitated with L243 obtained from interferon-γ-untreated cells. IFN, interferon; IP, immunoprecipitate; FAK, focal adhesion kinase; p-FAK, phospho focal adhesion kinase.

Ligation of human leukocyte antigen-DR molecules on fibroblasts with L243 results in the phosphorylation of focal adhesion kinase

To establish whether focal adhesion kinase is actually activated in fibroblasts upon stimulation with L243, interferon-γ-treated cells were first stimulated with L243 or with control IgG, and then the cells were lysed, followed by immunoprecipitation. As shown in Fig. 3, enhanced phosphorylation of focal adhesion kinase was actually observed 10 min following L243 stimulation, whereas the level of phosphorylation was weak in control IgG-stimulated cells. The amounts of total cellular focal adhesion kinase proteins did not change, regardless of the stimulation with L243 (Fig. 3, lower panel). Thus, the results indicate that human leukocyte antigen-DR-associated focal adhesion kinase is activated in fibroblasts upon stimulation with L243.

Luteolin, but not quercetin, suppresses phosphorylation of focal adhesion kinase, but does not influence the phosphorylation of ERK, p38 and c-jun N-terminal kinase MAPKs and c-jun

We next wondered whether any reagent suppressed the phosphorylation of focal adhesion kinase. As certain flavonoids have been reported to inhibit focal adhesion kinase activity in different cell types, we studied the effects of flavonoids, such as luteolin and quercetin, on the phosphorylation of focal adhesion kinase. As luteolin and quercetin have been suggested to suppress the activities of other signaling molecules in other cell types, we also tested the effects of these reagents on the activities of other kinases. Luteolin, but not quercetin, suppressed the phosphorylation of focal adhesion kinase (pY397) in a dose-dependent manner (Fig. 4A). However, luteolin did not affect the phosphorylation of ERK, p38 and c-jun N-terminal kinase MAPKs, and c-jun (Fig. 4B). In some kinases, such as p38, c-jun N-terminal kinase and c-jun, slightly enhanced phosphorylation was observed. Additionally, 100 μM luteolin resulted in the loss of both ERK and p-ERK. Cell viability was not influenced by the experimental concentration of luteolin, as judged by the Trypan Blue dye-

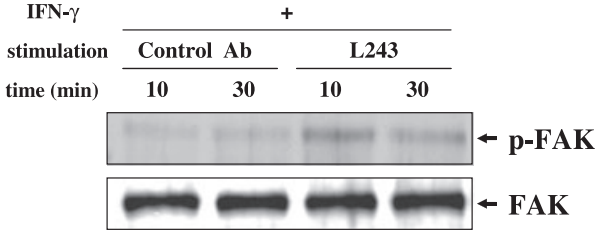

Fig. 3. Focal adhesion kinase is phosphorylated upon stimulation with L243. Interferon-γ-treated cells were first stimulated either with L243 or with control IgG for the time period indicated. The cells were lysed, and the lysates were immunoprecipitated. Ten minutes after stimulation with L243, larger amounts of the phosphorylated form of focal adhesion kinase were observed. The lower panel indicates the total focal adhesion kinase proteins detected from the total cell lysates in each sample. Ab, antibody; IFN, interferon; FAK, focal adhesion kinase; p-FAK, phospho focal adhesion kinase.

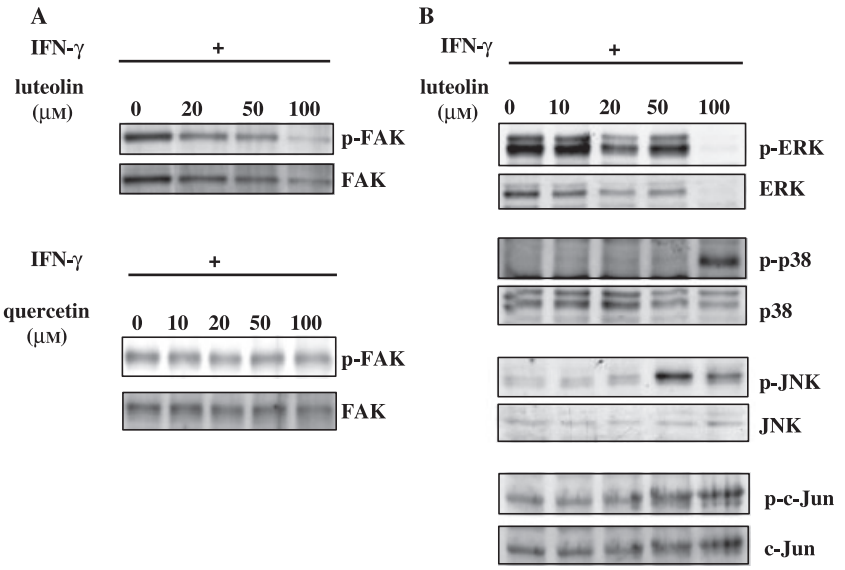

Fig. 4. Luteolin, but not quercetin, inhibits focal adhesion kinase phosphorylation (A), and the effects of luteolin on the phosphorylation of extracellular signal-regulated kinase, p38, c-jun N-terminal kinase, mitogen-activated protein kinases and c-jun (B). The effects of luteolin and quercetin on the phosphorylation of focal adhesion kinase were examined (A). Luteolin suppressed the phosphorylation of focal adhesion kinase in a dose-dependent manner. The effects of luteolin on the phosphorylation of mitogen-activated protein kinases and c-jun were examined (B). The membrane was first probed with antibody against extracellular signal-regulated kinase, p38, c-jun N-terminal kinase and c-jun, respectively. Then, the same membrane was stripped and reprobed with antibody reacting against the phosphorylated form of each molecule. Luteolin did not suppress the phosphorylation of extracellular signal-regulated kinase, p38, c-jun N-terminal kinase mitogen-activated protein kinases or c-jun. ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; IFN-γ, interferon-γ; JNK, c-jun N-terminal kinase; p-c-jun, phosphorylated c-jun; p-ERK, phosphorylated extracellular signal-regulated kinase; p-FAK, phosphorylated focal adhesion kinase; p-JNK, phosphorylated c-jun N-terminal kinase; p-p38, phosphorylated p38.

exclusion assay. Based on these results, we utilized luteolin for subsequent experiments.

Luteolin inhibits the production of monocyte chemoattractant protein 1, interleukin-6 and RANTES in fibroblasts stimulated with L243

Finally, we attempted to establish the effects of luteolin on L243-stimulated monocyte chemoattractant protein 1, interleukin-6 and RANTES production in fibroblasts. We treated the cells with different concentration of luteolin for 48 h and then stimulated the cells with either L243 or control IgG. Sixteen hours after stimulation, the culture supernatants were collected and the amounts of RANTES, monocyte chemoattractant protein 1 and interleukin-6 were measured. The results are shown in Fig. 5. Luteolin suppressed L243-induced production of RANTES, monocyte chemoattractant protein 1 and interleukin-6 in a dose-dependent manner.

Discussion

The findings of the present study can be summarized as follows: human leukocyte antigen-DR molecules directly associate with focal adhesion kinase in fibroblasts; focal adhesion kinase can mediate human leukocyte antigen-DR-induced signals into the cells; certain flavonoids (such as luteolin) suppress focal adhesion kinase phosphorylation; and luteolin suppresses human leukocyte antigen-DR-induced cytokine and chemokine production from fibroblasts. Therefore, focal adhesion kinase structurally associates with human leukocyte antigen class II molecules and actually mediates human leukocyte antigen class II-induced signaling, which leads to the secretion of cytokines.

Although it is well accepted that focal adhesion kinase signals play an important role in cell migration, proliferation and differentiation, no reports are available regarding the selective inhibitors for focal adhesion kinase. Therefore, in this study we searched the literature to find possible inhibitors for focal adhesion kinase, and chose quercetin and luteolin because these two reagents were the only reported inhibitors for focal adhesion kinase (5). The results indicated that quercetin did not influence the phosphorylation status of focal adhesion kinase in fibroblasts, whereas luteolin inhibited the phosphorylation of focal adhesion kinase in a dose-dependent manner. However, luteolin has been suggested to affect other signaling molecules, such as ERK, p38 MAPK and c-jun N-terminal kinase activity, in the murine macrophage cell line, RAW 264, and in human cultured mast cells, vascular smooth muscle cells, Hep G2 cells, colon epithelial cells and human umbilical vein endothelial cells (6–11). Additionally, luteolin has recently been reported to suppress the phosphorylation of c-jun without affecting MAPK activity, thereby suppressing the activity of the transcription factor, activator protein-1, in the basophilic cell line, KU812 (12). Therefore, we tested the possibility that luteolin might also influence the phosphorylation of signaling molecules, such as MAPK and c-jun, in gingival fibroblasts. However, no apparent inhibition on the phosphorylation of ERK, p38, c-jun N-terminal kinase and c-jun were observed. Additionally, luteolin even enhanced the phosphorylation of some signaling molecules, such as p38, p-c-jun N-terminal kinase and c-jun. We thus speculate that the effects of luteolin could differ from cell type to cell type.

In our previous study, we reported that the ligation of human leukocyte antigen-DR molecules with L243 resulted in the phosphorylation of c-jun N-terminal kinase-2 in gingival fibroblasts, and that the inhibition of c-jun N-terminal kinase activity with

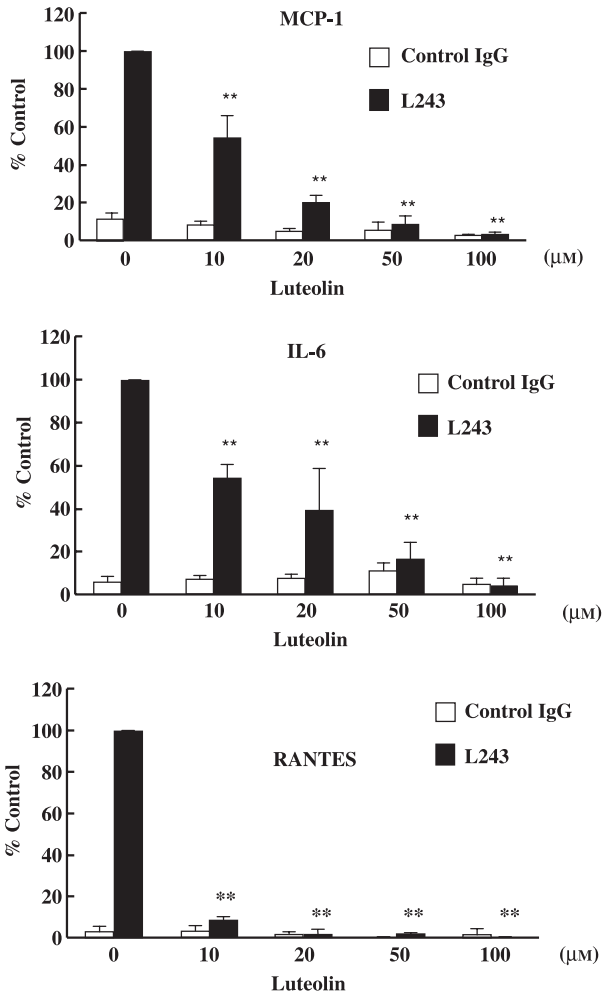

Fig. 5. Luteolin suppresses human leukocyte antigen-DR-induced cytokine production in fibroblasts. The cells were first treated with or without the indicated concentration of luteolin for 48 h, and then stimulated with either L243 or control IgG. Sixteen hours following stimulation, culture supernatants were collected and the concentrations of monocyte chemoattractant protein 1, interleukin-6 and RANTES were measured. Luteolin dose dependently suppressed monocyte chemoattractant protein 1, interleukin-6 and RANTES production in fibroblasts stimulated with L243. Data are expressed as percentage production of each cytokine from the cells stimulated with L243 in the presence of luteolin against that from the cells cultured without luteolin. *, $p < 0.05$, and **, $p < 0.01$ (Student's *t*-test). IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein 1; RANTES, regulated upon activation, normal T-cell expressed and secreted.

specific c-jun N-terminal kinase inhibitor dose-dependently suppressed RANTES production (3). However, in our present study, luteolin did not influence the phosphorylation status of c-jun N-terminal kinase, although it inhibited RANTES production in fibroblasts. We therefore speculate that c-jun N-terminal kinase is a downstream signaling molecule of human leukocyte antigen-DR-induced focal adhesion kinase-mediated signals in fibroblasts. In fact, c-jun N-terminal kinase was previously reported to be one of the downstream signaling molecules of focal adhesion kinase (13,14). Additionally, enhanced phosphorylation of Raf was observed in fibroblasts when stimulated with L243 by protein microarray analyses, which was also reported to be located downstream of focal adhesion kinase signals (15). Therefore, human leukocyte antigen class II-induced signals, leading to RANTES production, may be mediated through focal adhesion kinase, followed by c-jun N-terminal kinase, whereas such signals leading to interleukin-6 and monocyte chemoattractant protein 1 production could also be mediated through focal adhesion kinase, but not followed by c-jun N-terminal kinase. In this case, other downstream signaling molecules of focal adhesion kinase might mediate such signals. Human leukocyte antigen class II-mediated signaling pathways in gingival fibroblasts, revealed to date, including our current results, are summarized in Fig. 6.

It is generally accepted that focal adhesion kinase plays an important role in cell migration. Therefore, we tested the possibility that L243 may promote cell migration for fibroblasts. However, we did not observe apparent chemotactic responses of the cells against L243, as judged by chemotaxis assay (data not shown). Recently, however, the ligation of human leukocyte antigen-DR on malignant melanoma cells by L243 has been reported to increase the phosphorylation of focal adhesion kinase, and the proliferative activity of such cells was greatly impaired when the human leukocyte antigen-DR molecules were stimulated with L243 (16). Based on this observation and the results of the present study, it is possible that human leukocyte antigen-DR molecules in both fibroblasts and epithelial cells associate with focal adhesion kinase. Also, human leukocyte antigen molecules belong to the immunoglobulin superfamily, and some family members, such as intercellular adhesion molecule-1 and CD146, have recently been reported to associate with focal adhesion kinase (17,18). Interestingly, the human leukocyte antigen class I molecule also appeared to associate with focal adhesion kinase in endothelial cells (19,20). Taken together, it is not surprising that human leukocyte antigen class II molecules on gingival fibroblasts structurally associate with focal adhesion kinase.

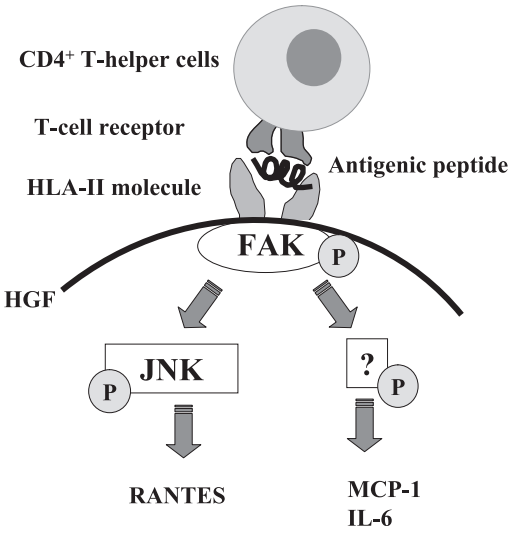

Fig. 6. Schematic presentation of the human leukocyte antigen class II-mediated signals in gingival fibroblasts. Formation of human leukocyte antigen class II–antigenic peptide-T-cell receptor complex between fibroblasts and $CD4^+$ T cells activates focal adhesion kinase signaling pathways. Focal adhesion kinase directly or indirectly activates c-jun N-terminal kinase, resulting in the production of RANTES, while at the same time focal adhesion kinase phosphorylatiuon results in the production of monocyte chemoattractant protein 1 and interleukin-6 via signaling molecules other than c-jun N-terminal kinase. FAK, focal adhesion kinase; HGF, human gingival fibroblast; HLA, human leucocyte antigen; JNK, c-jun N-terminal kinase; MCP-1, monocyte chemoattractant protein 1; P, phosphate; RANTES, regulated upon activation, normal T-cell expressed and secreted.

In this study, we found that luteolin suppressed focal adhesion kinase phosphorylation and inhibited human leukocyte antigen class II-induced cytokine production. This may partially account for the reported antioxidant and anti-inflammatory properties of luteolin (21). As luteolin is a flavonoid naturally occurring in certain plants, it could be useful for clinically reducing unfavorable inflammatory responses that lead to further tissue destruction, in the case of chronic periodontal disease and/or some autoimmune diseases, such as rheumatoid arthritis.

In conclusion, this study demonstrated that human leukocyte antigen class II molecules on gingival fibroblasts associate with focal adhesion kinase in nonprofessional antigen-presenting cells and in nonmalignant normal cells, such as gingival fibroblasts. Additionally, focal adhesion kinase appeared to transduce human leukocyte antigen class II-induced signals into the cells, leading to the production of cytokines such as interleukin-6, monocyte chemoattractant protein 1 and RANTES.

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