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CD23-mediated transport of IgE/immune complexes across human intestinal epithelium: role of p38 MAPK

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Tu, Yahong, and Mary H. Perdue. CD23-mediated transport of IgE/immune complexes across human intestinal epithelium: role of p38 MAPK. *Am J Physiol Gastrointest Liver Physiol* 291: G532–G538, 2006. First published; doi:10.1152/ajpgi.00524.2005.—We previously reported that CD23/FcεRII (low-affinity IgE receptor) is expressed on human intestinal epithelial cells and is responsible for transepithelial transport of IgE. In this study, we compared the transport of IgE with that of immune complexes in both the apical-to-serosal and the serosal-to-apical directions across HT29 epithelial cell layers and examined the effects of two p38 MAPK inhibitors, SKF86002 and SB203580, on the expression and function of CD23. Our study showed that both p38 MAPK inhibitors at 10 μM significantly inhibited constitutive and IL-4-upregulated CD23 protein expression in epithelial cells. Both inhibitors, in a concentration-dependent manner, also significantly reduced IgE binding and uptake into cells. Transepithelial transport of IgE and immune complexes across the epithelial barrier were similarly inhibited. IL-4 upregulated the phosphorylation and activity of p38 MAPK and the phosphorylation of the downstream substrate MAPKAP-2 (MK-2). The inhibitors exerted effects in the pathway post the p38 MAPK; SB203580 significantly inhibited the phosphorylation of MK-2. Our results indicate that CD23 expression in these human intestinal epithelial cells is mediated through the p38 MAPK pathway and that inhibition of p38 MAPK consequently interferes with the transport of IgE and immune complexes across the intestinal epithelial barrier.

low-affinity immunoglobulin E receptor; intestinal permeability; antigen

ALLERGIC DISEASES are the most common type of immune disorders and are increasing in incidence in the developed world (24). Food allergies affect 2–5% of adults and up to 8% of children (28). The symptoms of food allergies include nausea, vomiting, and diarrhea, as well as nonintestinal symptoms in the skin and airways. Currently, there are few effective treatments for these conditions apart from avoidance of offending foods. Elimination diets may result in lack of adequate nutrition, particularly in growing children. Therefore, it is important to understand the mechanisms involved in generating food allergic reactions to design novel therapeutic approaches.

An ingested antigen must cross the epithelium lining the intestinal tract to gain access to mucosal immune effector cells that are activated in intestinal anaphylactic reactions. A key immunoglobulin in allergies is IgE (31). There is evidence that in sensitized individuals, IL-4 is produced in excess and that IL-4 stimulates the production of IgE as well as expression of

its receptors (10, 11, 34). The high-affinity receptor FcεRI is expressed on mast cells (21), and cross-linking of mast cell-bound IgE by antigen results in activation of these cells with release of potent mediators (36). In the gut mucosa, the action of these mediators on structural cells, such as epithelial cells and smooth muscle cells, causes the symptoms of food allergies (40). Interfering with antigen ingress into the mucosa might be a strategy to limit the activation of effector cells and thus reduce allergic reactions.

Our previous studies (4) in rodent models of food allergy demonstrated that transepithelial uptake of antigen from the intestinal lumen was enhanced in sensitized animals compared with controls. This enhanced uptake was specific for the antigen to which the animal was sensitized and dependent on IgE (37). Subsequent studies (39) in mice provided evidence that CD23 (the low-affinity receptor for IgE) expressed on the apical membrane of intestinal epithelial cells (enterocytes) was mediating this effect. Human epithelial cells, both in tissues as well as cultured cell lines, also express CD23 (34). We recently reported (34) that the expression of human intestinal epithelial CD23 is regulated by IL-4. In that study, we also demonstrated CD23-mediated IgE uptake and bidirectional transport across human epithelium; CD23 antibody concentration-dependently reduced IgE transport across HT29 cultured epithelial layers grown on filter supports. Transfection of these epithelial cells with antisense oligonucleotides inhibited CD23 expression; this strategy also reduced the transepithelial transport of IgE.

In human monocytes, expression of CD23 protein was reduced by inhibitors to p38 MAPK (22). Specifically, inhibition of phosphorylation of p38 MAPK protein and cascading phosphorylations of downstream proteins, such as MAPKAP-2 (MK-2) by SKF86002 or SB203580, resulted in downregulated expression of CD23. These inhibitors were shown to reduce the surface expression of intact CD23, involving either transcriptional or translational regulation. The role of p38 MAPK in regulating epithelial CD23 expression has not been reported previously.

The current study was designed to continue our studies of the function of CD23 in human epithelial cells and to attempt to interfere with its function in an indirect manner. Although we had previously demonstrated CD23-mediated transport of IgE (34), we had not examined whether immune complexes can be carried across the intestinal barrier by this receptor. In this study, we used HT29 human epithelial cells as in our previous study (26), because they express CD23 in response to IL-4 (but do not develop decreased resistance) and form

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epithelial layers suitable for transport experiments. This study demonstrates that inhibition of p38 MAPK results in the downregulation of epithelial CD23 expression accompanied by reduced transepithelial transport of IgE and, more importantly, of IgE-antigen immune complexes across the epithelial barrier.

MATERIALS AND METHODS

Chemicals and antibodies. The CD23 antibody used in the study was mouse anti-human CD23, clone Tu1 (NovoCastra Laboratories, Newcastle upon Tyne, UK). Chimeric human IgE raised against 4-hydroxy-3-nitrophenylacetyl (NP) was a product of Serotec (Oxford, UK). NP(16)-ovalbumin (OVA) was purchased from Biosearch Technologies (Novato, CA). Rabbit polyclonal anti-human IgE antibody was purchased from DAKO Diagnostics (Mississauga, Ontario, Canada). Monoclonal anti-OVA antibody was purchased from Sigma-Aldrich (St. Louis, MO). All antibodies to phosphorylated and non-phosphorylated p38 MAPK, MK-2, and a kit for measuring p38 MAPK activity were from Cell Signaling Technology (Beverly, MA). Monoclonal anti- β -actin was a product of Sigma-Aldrich. Recombinant human IL-4 was from R&D Systems (Minneapolis, MN). The p38 MAPK inhibitors SKF86002 and SB203580 were products of Calbiochem-Novabiochem (San Diego, CA).

Epithelial cell culture. HT29-C1 19A human intestinal epithelial cells were used for these studies, because we had previously determined (34) that of the three human epithelial cell lines tested, these HT29 cells produced the most consistent results, and the nonspecific permeability of HT29 epithelia was not affected by IL-4. The cells were cultured in McCoy's 5A modified medium without glucose (Invitrogen Canada, Burlington, Ontario, Canada), supplemented with 5% FBS, 0.0375% sodium bicarbonate, 0.2 mM L-glutamine, and penicillin/streptomycin, in an atmosphere of 5% CO₂ at 37°C. The cells were split every 5 days, and media was changed every 2–3 days. Cells (5×10^5) were seeded into wells of 24-well plates, onto coverslips, or onto Transwell filters (Corning, Corning, NY) for transport studies. In the latter case, the cells were grown either on the normal or reverse side of the filter, which was then cultured in a conical tube with 25 ml media. The filter was returned to the Transwell compartment when the transepithelial resistance was at least 400 Ω /cm² (~7 days), measured by an Ohm meter with chopstick electrodes (Millicell-RES; Millipore, Bedford, MA). Under these conditions, the cells formed differentiated monolayers. CD23 expression was stimulated by IL-4 (10 ng/ml) added 24 h before the experiment.

CD23 protein measurement by Western blot. Cells grown in plates were washed twice with cold PBS (pH 7.2) containing a cocktail of protease inhibitors (Sigma). The cells were harvested by scraping them into 500 μ l of cold lysis buffer (100 mM NaCl, 10 mM Tris·HCl, 2 mM EDTA, 1.8% Triton X-100, and protease inhibitors) (pH 7.8). The total protein in the supernatant of the cell lysate was measured by the Bio-Rad protein assay (Bio-Rad Laboratories, Mississauga, ON, Canada). Then, 80 μ g of protein for each sample were migrated on a 10% acrylamide gel and analyzed by SDS-PAGE. The proteins were transferred electrophoretically onto a nitrocellulose membrane and incubated with anti-human CD23 antibody (1:500) for 48 h at 4°C. After being washed with PBS, horseradish peroxidase (HRP)-conjugated secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) was added. An enhanced chemiluminescence detection method was used to detect the target CD23 protein using a LumiGlo substrate kit (KP Laboratories, Gaithersburg, MD) and X-ray film. The integrated intensity for the bands was quantified by scanning densitometry.

IgE binding/uptake into epithelial cells. IgE uptake into epithelial cells was determined using HT29 cells cultured on coverslips for 3 days. Cells were treated with IL-4 for 24 h and/or p38 MAPK inhibitors added 2 h before IL-4. Cells were then incubated for 90 min

at 37°C with 10 μ g/ml IgE [dissolved in IgE binding buffer, (in mM) 10 Tris·HCl (pH 7.35), 140 NaCl, 2 CaCl₂, and 1 MgCl₂, with 1.8 g/l glucose] (30). The cells were washed three times with cold PBS, including protease inhibitors, and then fixed with 50% acetone buffered in PBS for 15 min. After being washed, endogenous peroxidase activity was eliminated by 3% H₂O₂ for 15 min. The cells were blocked with 0.1 M Tris·HCl, 0.15 M NaCl, and 0.5% blocking reagent; pH 7.5 (TNB buffer) for 30 min. IgE was recognized by a rabbit polyclonal antibody against the ϵ -chain of human IgE (1:500) added to the cells for 30 min. The signal was amplified using streptavidin-HRP and tyramide fluorescein (TSA kit; NEN Life Science Products, Boston, MA). Images were observed under the confocal microscope and analyzed using LSM510 software.

Transepithelial transport of IgE or immune complexes. IgE transport across epithelial monolayers (after pretreatment with IL-4 and/or p38 MAPK inhibitors) was performed in Transwell compartments by adding 0.2 ml of the IgE transport solution (0.4 μ M IgE in binding buffer) to either the apical or basal compartment (depending on whether the cells had been grown on the normal or reverse side of the filter). After a 2-h incubation at 37°C, the media from the opposite compartment was collected at 4°C. IgE was measured by Western blot using HRP-conjugated rabbit anti-human IgE antibody (1:20,000), details as for CD23. (The 2-h time was selected to accumulate enough IgE to measure accurately.)

To form immune complexes, NP-OVA (0.5 μ M) was incubated with IgE anti-NP (0.2 μ M) for 60 min. The complex was added to the apical or basal compartment. Media in the opposite compartment of the monolayer was collected for Western blot analysis using a secondary antibody to OVA (1:10,000) to detect the NP-OVA. Two bands were detected. The band with the larger molecular weight (>200 kDa), indicative of the complex, was quantified by densitometry.

Measurement of phosphorylated p38 MAPK. Phosphorylated p38 MAPK was analyzed by Western blot analysis of plate-grown cells after specific treatments. The cells were lysed in PBS containing (in mM) 20 HEPES (pH 7.5), 0.2 EDTA, 5 DTT, 1 PMSF, and 1.2 Na₃VO₄ added to the lysis buffer. Then cells were scraped off the culture dishes and centrifuged at 10,000 g for 30 min at 4°C. The supernatant was mixed with an equal volume of the same buffer including 40% glycerol (12). The samples were loaded onto 10% SDS-PAGE and transferred to a nitrocellulose membrane. The specific rabbit anti-phospho-p38 MAPK antibody against Thr¹⁸⁰/Tyr¹⁸² sites, which does not crossreact with phosphorylated threonine/tyrosine of ERK 1/2 or JNK (Cell Signaling Technology, bulletin no. 9211), was incubated with the protein on the membrane at a dilution of 1:1,000 for 48 h at 4°C. The secondary HRP-conjugated anti-rabbit antibody was incubated with the membrane for 2 h at a 1:40,000 dilution. After the specific signal was captured, the membrane was stripped by 0.1 M acetic acid in PBS for 1 h. The membrane was incubated again with nonphosphorylated p38 MAPK antibody. This signal was used as an internal reference to semiquantify the bands for each loading sample. The ratio of the band-integrated intensity for the phospho-p38 MAPK to the band-integrated intensity for the nonphospho-p38 MAPK was used to indicate the change in phosphorylation.

Phospho-p38 MAPK activity. The activity of phospho-p38 MAPK was performed using a p38 MAPK assay (Cell Signaling Technology). Briefly, the monoclonal phosphospecific antibody to p38 MAPK (Thr¹⁸⁰/Tyr¹⁸²) was employed to selectively immunoprecipitate active phospho-p38 MAPK from cell lysates. The resulting immunoprecipitate was then incubated with activating transcription factor-2 (ATF-2) fusion protein in the presence of ATP and kinase buffer. This allowed the immunoprecipitated active p38 MAPK to phosphorylate ATF-2 in vitro. The phosphorylation of ATF-2 at Thr⁷¹ was measured by Western blot using an anti-phospho-ATF-2 (Thr⁷¹) antibody. Thr⁷¹ of ATF-2 is a major ATF-2 phosphorylation site required for transcriptional activity (20).

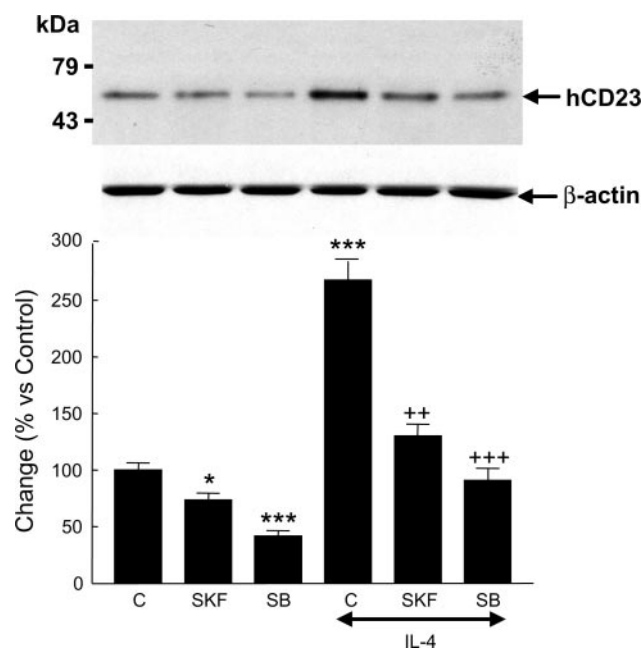


Fig. 1. Inhibition of constitutive and IL-4-enhanced CD23 protein expression by p38 MAPK inhibitors. HT29 human intestinal epithelial cells (3×10^6) were cultured in 24-well plates for 3 days. Then cells were cultured for an additional 24 h with IL-4 (10 ng/ml) or vehicle. Each p38 MAPK inhibitor, SKF86002 (SKF) or SB203580 (SB), was added 2 h before IL-4 or vehicle. CD23 protein expression is indicated by Western blots. Integrated intensity was determined by scanning densitometry using β -actin as an internal control. Bars indicate the percentage change compared with control (C) in the absence of IL-4. Values are means $\pm$ SE; $n = 5$ separate experiments. * $P < 0.02$, *** $P < 0.0001$ vs. control. ++ $P < 0.001$, +++ $P < 0.0001$ vs. IL-4 alone.

Determination of the phosphorylation for MK-2. The phospho-MK-2 was also measured by Western blot. After treatments, the cells were immediately scraped off the culture dishes in a $1 \times$ SDS sample buffer and sonicated for 10–15 s. The samples were heated for 5 min at 100°C and centrifuged at $10,000 \text{ g}$ for 5 min. Protein for each

sample was loaded onto 10% SDS-PAGE and transferred to the nitrocellulose membrane. The protein on the membrane was incubated with a specific rabbit anti-phospho-MK-2 (Thr³³⁴) antibody overnight at 4°C , in a dilution of 1:2,000. This antibody detected MK-2 only when phosphorylated at Thr³³⁴. The signal elicited from the protein was visualized by HRP-conjugated secondary antibody and enhanced chemiluminescence. Then the antibodies were stripped (by adding 0.1 M acetic acid in PBS for 30 min followed by three washes in PBS), and the protein on the membrane was further incubated with rabbit anti-nonphospho-MK-2 antibody, which served as an internal control. The phosphorylation of MK-2 was indicated as the ratio of the phospho-MK-2 over the nonphospho-MK-2.

Statistical analysis. Statistical analysis was performed using a two-tail Student's t -tests or ANOVA. A P value < 0.05 was considered to be significant.

RESULTS

Effect of p38 MAPK inhibitors on CD23 expression. We previously showed (5) that CD23 protein is constitutively expressed in epithelial HT29 cells and upregulated by IL-4. Here, we examined the effect of two p38 MAPK inhibitors, SKF86002 and SB203580. Both inhibitors at $10 \mu\text{M}$ significantly inhibited the constitutive expression of CD23 protein in human intestinal epithelial cells (Fig. 1). In addition, both inhibitors (added 2 h prior to IL-4) resulted in a significant diminution of the IL-4-enhanced CD23 protein expression. SB203580 was more effective, resulting in $\sim 50\%$ decrease in constitutive expression; IL-4-stimulated expression was reduced to $\sim 33\%$ by SKF86002 compared with $\sim 50\%$ by SB203580.

Effect of p38 MAPK inhibitors on IgE uptake. Immunohistochemistry of IgE uptake into epithelial cells was conducted using IL-4-treated cells. Confocal imaging showed that IgE was internalized into epithelial cytoplasmic compartments (Fig. 2). Both of the p38 MAPK inhibitors reduced fluorescence in a concentration-dependent manner, with partial inhibition at $10 \mu\text{M}$ and virtually complete elimination at $20 \mu\text{M}$.

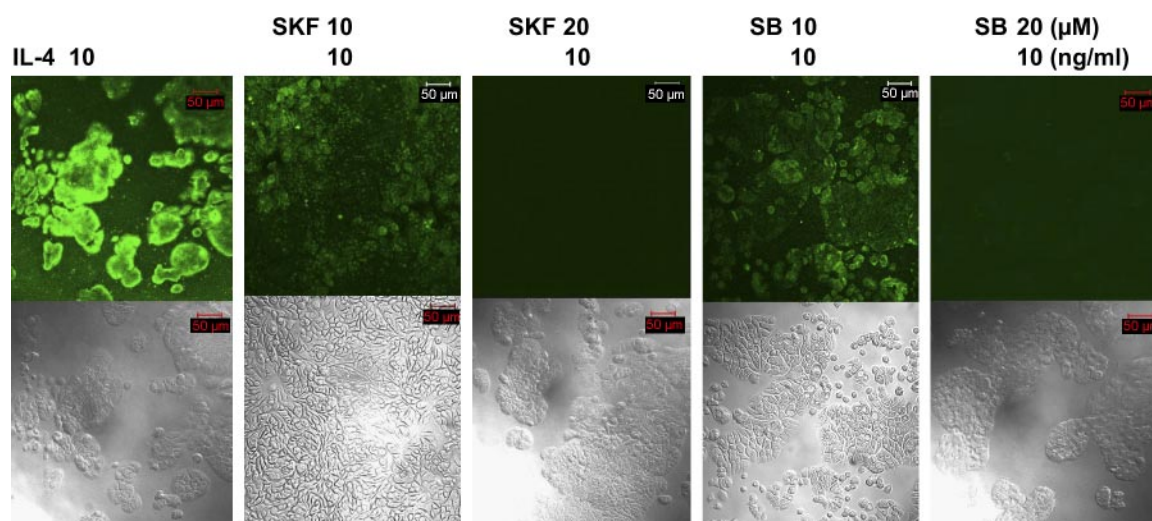


Fig. 2. Reduction of IgE binding and uptake by p38 MAPK inhibitors. HT29 cells were grown on coverslips for 3 days. The cells were cultured for an additional 24 h with IL-4 (10 ng/ml). Each p38 MAPK inhibitor, SKF or SB at 10 or $20 \mu\text{M}$, was added 2 h before IL-4. The culture media with the reagents were discarded. Then IgE (in IgE binding buffer), was added for 90 min at an atmosphere of 37°C , $5\% \text{ CO}_2$, allowing for IgE binding and uptake. After 3 washes, the cells were fixed with PBS-buffered 50% acetone. Endogenous peroxidase activity was eliminated by H_2O_2 . A polyclonal rabbit anti-IgE ϵ -chain antibody was then incubated with the cells, and the signal was amplified using streptavidin-HRP and tyramide fluorescein. The upper panels are laser confocal images showing that the p38 MAPK inhibitors reduce IgE binding/uptake in a concentration-dependent manner. The lower panels are phase-contrast images.

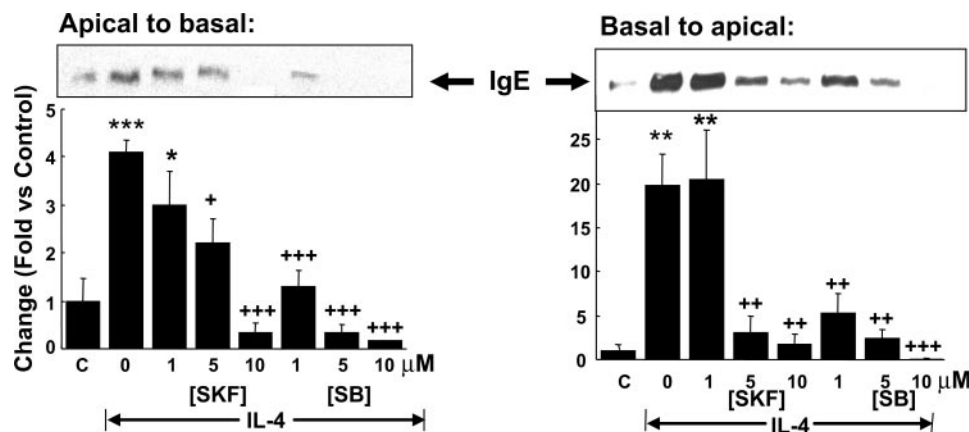


Fig. 3. Effects of p38 MAPK inhibitors on transepithelial IgE transport. HT29 cells were grown on transwell filters for 7 days until transepithelial resistance was $>400 \Omega/\text{cm}^2$. Monolayers with identical resistance were cultured for an additional 24 h with IL-4 (10 ng/ml) or vehicle. Each p38 MAPK inhibitor, SKF or SB, was added 2 h before IL-4 or vehicle. IgE (0.4 μM) was added to either the apical or basal compartment. After 2 h, the media in the opposite compartment was collected for Western blot analysis. The band intensity of the transported IgE was quantified by densitometry. Bars indicate the fold change compared with C in the absence of IL-4. Values are means $\pm$ SE; $n = 4$ separate experiments. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.002$ vs. control. $^+P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ vs. IL-4 alone.

Effect of p38 MAPK inhibitors on transepithelial transport of IgE. Transepithelial transport of IgE across IL-4-treated cells occurred in both directions, from the apical-to-basal side of the epithelial monolayer and also from the basal-to-apical side of the monolayer (Fig. 3). However, the lumenally directed transport (from basal-to-apical) was relatively greater, being approximately five times that in the opposite direction. The p38 MAPK inhibitors dose-dependently inhibited transepithelial IgE transport in both directions. At 10 μM , particularly for SB203580, transport of IgE was negligible.

Effect of the p38 MAPK inhibitors on the transepithelial transport of immune complex. Transepithelial transport of the IgE immune complex across IL-4-treated cells also occurred in both directions (Fig. 4). However, in this case, the serosally directed transport (from apical-to-basal) was relatively greater than the lumenally directed transport. Again, the p38 MAPK

inhibitors dose-dependently inhibited transepithelial immune complex transport in both directions.

Alteration of the p38 MAPK signal transduction pathway by IL-4. Figure 5A, shows that IL-4 treatment (10 ng/ml for 10 min) increased the phosphorylation of p38 MAPK in epithelial cells. Phosphorylation was inhibited by the p38 MAPK inhibitors at 10 μM (Fig. 5B). The inhibitors had no effect on phosphorylation in the absence of IL-4 (data not shown).

Because MK-2 is a key downstream factor in the p38 MAPK pathway, we determined the phosphorylation of this protein after IL-4 stimulation. Figure 6A shows a time-dependent change in phosphorylated MK-2. Phosphorylation of the protein was evident at 15 min after treatment with IL-4 and lasted for ~ 6 h. Figure 6B, shows the inhibitory effect of the p38 MAPK inhibitor SB203580 (10 μM) on the IL-4-enhanced phosphorylation of the MK-2. In this study, both phosphory-

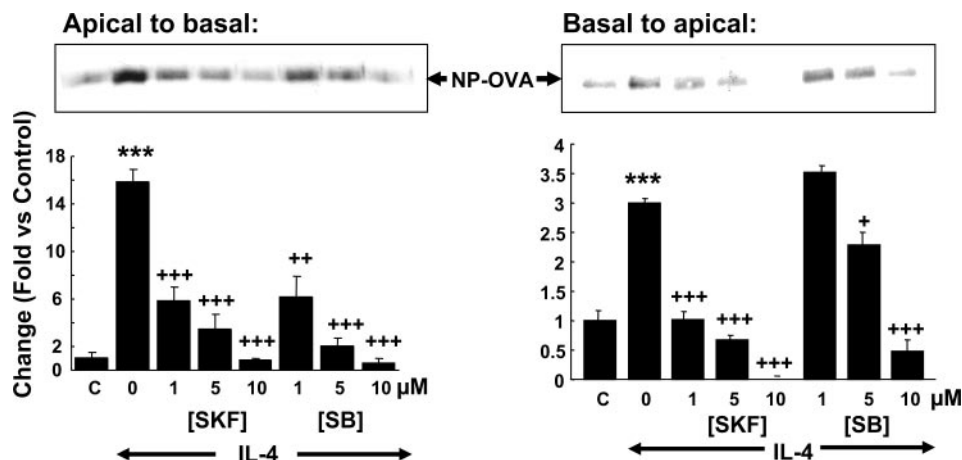


Fig. 4. Inhibition of specific antigen transport across HT29 monolayer by p38 MAPK inhibitors. HT29 cells were grown on transwell filters for 7 days until transepithelial resistance was $>400 \Omega/\text{cm}^2$. Monolayers with identical resistance were cultured for an additional 24 h with IL-4 (10 ng/ml) or vehicle. Each p38 MAPK inhibitor, SKF or SB, was added 2 h before IL-4 or vehicle. Immune complexes, formed by incubating 4-hydroxy-3-nitrophenylacetyl (NP)-ovalbumin (OVA; 0.5 μM) with IgE anti-NP (0.2 μM) for 60 min, were added to either the apical or basal compartment. After 2 h, the media in the opposite compartment was collected for Western blot analysis. The band intensity of the large molecular mass OVA complex was quantified by densitometry. Bars indicate the fold change compared with C in the absence of IL-4. Values are means $\pm$ SE; $n = 4$ separate experiments. $^{***}P < 0.001$ vs. control. $^+P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ vs. IL-4 alone.

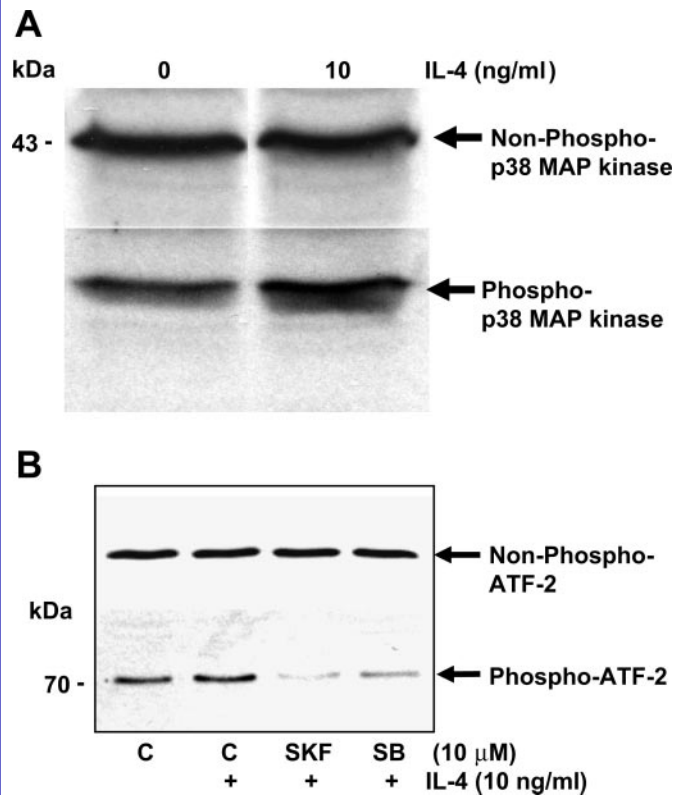


Fig. 5. A: effect of IL-4 on phosphorylation of p38 MAPK. HT29 cells were cultured in plates. After treatment with IL-4 (10 ng/ml) or vehicle for 10 min, the cells were lysed in an ice-cold lysis buffer and scraped off the plates. Western blot analysis was performed. After the membrane was with specific antibody to phosphorylated p38 MAPK, the image was visualized. The membrane was stripped with 0.1 M acetic acid in PBS and further incubated with an antibody against the nonphosphorylated p38 MAPK. The result shown is representative of 3 separate experiments. B: effect of p38 MAPK inhibitors on MAPK activity. HT29 cells were cultured in plates. Each p38 MAPK inhibitor, SKF or SB, was added for 2 h before IL-4 or vehicle. After incubation with IL-4 (10 ng/ml) or vehicle for 10 min, the cells were lysed and the protein was extracted. The phospho-p38 MAPK activity was measured using a p38 MAPK assay as described in the MATERIALS AND METHODS. The result shown is representative of 3 separate experiments.

lated and nonphosphorylated MK-2 were determined. The percentage of the ratio of phosphorylated MK-2 over nonphosphorylated kinase was $100 \pm 9.8\%$ for the control ($n = 5$) and $163 \pm 19.9\%$ ($n = 5$) for IL-4-treated cells ($P < 0.03$ vs. control). The p38 MAPK inhibitor SB203580 significantly inhibited the effect of the IL-4 on phosphorylation, reducing the percentage to $90 \pm 12\%$ ($n = 5$; $P < 0.02$ vs. IL-4 alone). Although SKF86002 inhibited the percentage from $163 \pm 19.9\%$ of the IL-4 treatment to $119 \pm 30.7\%$ of the IL-4 plus SKF86002 ($n = 5$), there was no significant difference between the two groups.

DISCUSSION

We previously reported (34) that CD23, the low-affinity receptor for IgE, is expressed by human enterocytes. In addition, we showed that CD23 functions to bind, take up, and transport IgE across intestinal epithelial monolayers. Here, we showed that IgE immune complexes are also transported by epithelial CD23 in a bidirectional manner and that IgE is preferentially transported toward the lumen, whereas immune

complexes are preferentially transported in the opposite (into the mucosa) direction across HT29 epithelial cell layers. We provided evidence that p38 MAPK is involved in the pathway-regulating expression of CD23 and that inhibition of this enzyme can virtually eliminate the transepithelial transport of both IgE and its immune complexes.

For many years we had observed that food antigen-induced changes in intestinal function (e.g., ion secretion) in sensitized rodents occurred extremely rapidly (within minutes) (4). The effector cells were shown to be mast cells located in the subepithelial mucosa (5, 9). Normally, the epithelial barrier (single cell layer of conjoined cells lining the intestinal tract) restricts penetration into the mucosa of antigen-sized molecules (13). To determine the route and rate of antigen uptake across the epithelial barrier, we sensitized rats to HRP and two wk later challenged intestinal tissues with HRP added to the luminal side of tissues mounted in Ussing chambers. HRP antigen was identified in the lamina propria at 2 min ($20\times$ faster than normal), and by 5 min there was evidence of mast cell activation associated with ion secretion (indicated by

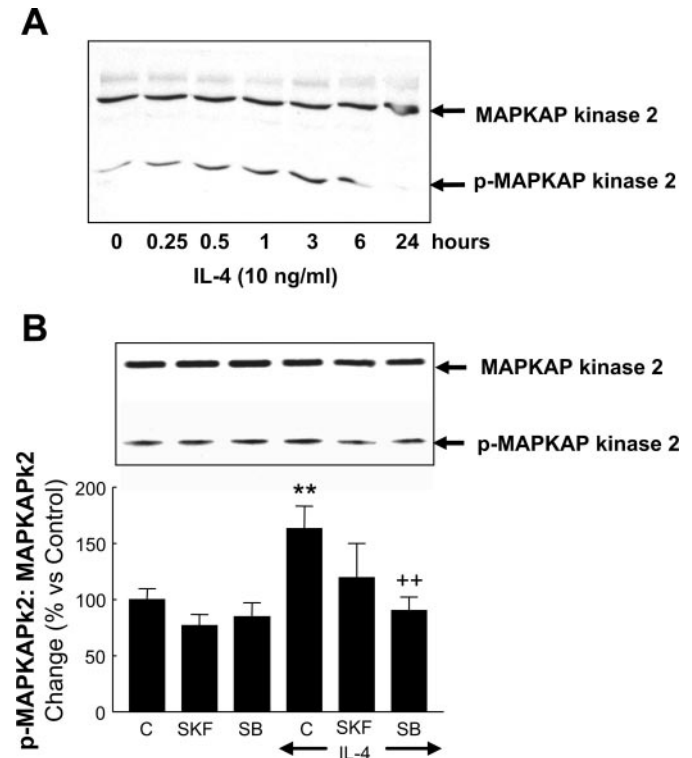


Fig. 6. A: effect of IL-4 on phosphorylation of MAPKAPK-2. HT29 cells were grown in plates and treated with IL-4 (10 ng/ml) for different times. Western blot analysis was performed. After incubating the membrane with specific antibody to phosphorylated MAPKAPK-2, the image was visualized. The membrane was stripped with 0.1 M acetic acid in PBS and further incubated with an antibody against the nonphosphorylated MAPKAPK-2. The result shown is representative of 3 separate experiments. B: effect of p38 MAPK inhibitors on phosphorylation of MAPKAPK-2. HT29 cells were cultured in plates. Each p38 MAPK inhibitor, SKF or SB, was added for 2 h before IL-4 or vehicle. After incubation with IL-4 (10 ng/ml) or vehicle for 10 min, the cells were lysed and the protein was extracted. Western blot analysis was performed as described in MATERIALS AND METHODS. Bars indicate the ratio of phosphorylated MAPKAPK-2 compared with nonphosphorylated MAPKAPK-2, expressed as percent change compared with C in the absence of IL-4. Values are means $\pm$ SE; $n = 5$ separate experiments. $**P < 0.01$ vs. control, $++P < 0.01$ vs. IL-4 alone.



elevated short-circuit current) (3, 8). Because the accelerated responses were specific for the antigen to which the animal had been sensitized (4, 37), we postulated the involvement of an immunoglobulin recognition mechanism. Passive sensitization studies, in which IgE was depleted from immune serum transferred into naïve animals, demonstrated that IgE was required for enhanced transepithelial antigen uptake (39). We next showed that the enhanced antigen uptake was significantly inhibited by anti-CD23 antibody and was not present in CD23^{-/-} mice (39). Expression of CD23 mRNA and proteins (the classic b isoform as well as 2 unique splice variants) in mouse enterocytes was identified to be regulated by IL-4 (41). Taken together, these studies implicated CD23 in the transport of IgE-antigen immune complexes across the epithelial barrier into the body.

In humans, CD23 expression has been demonstrated in epithelial cells as well as a host of immune/inflammatory cells (1, 15, 27, 33–35, 38). Intestinal CD23 expression was identified before birth, at ~16 wk of gestation (19). In enterocytes, CD23 protein was visualized on both the brush border luminal membrane and on the basolateral membrane (23, 34), suggesting that IgE or IgE immune complexes may be transported in both directions across the epithelium. In fact, we demonstrated bidirectional transepithelial IgE transport in our previous study (34), but we did not closely examine the relative amount of IgE transported in either direction. Here, we noted significantly greater transepithelial transport of IgE toward the lumen than in the opposite direction, whereas the opposite was true for immune complexes. Different isoforms/splice variants of CD23 are likely responsible for the preferential binding and transepithelial transport of IgE vs. its complexes (23, 41). This is an area of ongoing investigation.

Excessive IgE has been measured in the luminal contents/feces of food allergic individuals (6, 29), and it was suggested that IgE in the lumen is produced locally in the gastrointestinal mucosa (17). Whether produced in the mucosa or derived from the circulation, CD23-mediated IgE transport into the lumen would be a mechanism for clearance/secretion of the immunoglobulin. In studies of nematode infection in rats (25), excess IgE was also demonstrated in the gastrointestinal tract, where it was postulated to be involved in the host defense mechanism, that is, rapid expulsion of the parasite from an immunized host. Therefore, it is not surprising that our study indicated greater transport of IgE toward the lumen than in the opposite direction, whereas there was enhanced transport of immune complexes from the lumen toward the basal side of the monolayer (the physiologically relevant direction, because mucosal mast cells normally reside under the epithelium). The fate of CD23-bound IgE after transport is not clear. It might remain associated with its receptor or, because the receptor has relatively low affinity, be released into the lumen where it would be free to form immune complexes with ingested antigens. In this form, the conformation of the IgE molecule might be somewhat altered. It is possible that different splice variants of CD23b are expressed in human enterocytes and are responsible for the binding and transport of IgE vs. immune complexes. Different forms of CD23 may be directed differentially toward the basal or apical membrane. Studies need to address this issue.

In this study, we determined that the p38 MAPK inhibitors SKF86002 and SB203580 reduced the expression of CD23 in

intestinal epithelial cells and inhibited its function. Both constitutive and IL-4-stimulated expression was inhibited, similar to the results of Marshall et al. (22) in monocytes. We used IL-4-treated epithelial cell monolayers for all the uptake/transport experiments, because IL-4 is elevated in allergic conditions, making that the more relevant situation. Both inhibitors caused a concentration-dependent reduction in uptake of IgE into the cells. In addition, transepithelial transport of either IgE or immune complexes was significantly diminished by the p38 MAPK inhibitors. With respect to transport, p38 MAPK inhibition was concentration dependent and resulted in virtual elimination of transport at 10 μ M. The inhibition was similar, regardless of whether IgE was free or complexed to its antigen, and did not differ for either direction of transport (although there were minor differences in the effective concentrations, possibly due to altered CD23 protein conformation). This suggests that the inhibition of function was indirect and due to reduced expression of CD23.

IL-4 has been shown to upregulate CD23 expression in murine lymphocytes (7) and rodent and human intestinal epithelial cells (22, 34, 41). IL-4 also induces activation of p38 MAPK in murine B- and T-lymphoid cell lines (14). In addition, Marshall et al. (22) showed in monocytes that IL-4 upregulated CD23 expression, and those inhibitors for p38 MAPK reduced the IL-4-increased CD23 protein expression. Here, we identified that IL-4 increased the phosphorylation of the p38 MAPK in epithelial cells but had no effect on the nonphosphorylated p38 MAPK enzyme. Neither inhibitor alone had any effect on the phosphorylation of p38 MAPK. This is in accordance with the theory that both inhibitors are acting at a step downstream from the kinase (18). We found that the activity of p38 MAPK with respect to phosphorylation of ATF-2 was upregulated by IL-4 and was inhibited by SKF86002 or SB203580 at 10 μ M. Sundstrom et al. (32) also reported that p38 MAPK inhibitors attenuated stem cell factor-induced p38 MAPK activation cascade in a murine mast cell line. MK-2 is a substrate in the downstream signaling pathway of p38 MAPK. In this study, we observed that IL-4 stimulated the phosphorylation of MK-2, but did not alter the level of nonphosphorylated MK-2. Therefore, this result suggests that the IL-4-enhanced expression of CD23 involves signal transduction downstream of p38 MAPK.

In summary, our study indicates that inhibition of p38 MAPK inhibits the expression and function of CD23 in HT29 human intestinal epithelial cells. The p38 MAPK inhibitors exerted concentration-dependent inhibitory effects on the transport of IgE and IgE-antigen immune complexes across the intestinal barrier. We confirmed the involvement of the p38 MAPK signal transduction pathway. Recent publications (2, 16, 42) have provided evidence that the p38 MAPK pathway is involved in intestinal barrier damage after ischemia-reperfusion injury and after burn injury; inhibitors for p38 MAPK improved the integrity of the intestinal barrier and reduced the production of proinflammatory cytokines after damage. Because our studies were limited to only one line of cultured epithelial cells, we cannot extrapolate to all in vivo situations. However, our findings taken together with those of others suggest that p38 MAPK inhibitors deserve further study, because they may be potential candidates for the treatment of the food allergic conditions.



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