CD23-Dependent Transcytosis of IgE and Immune Complex across the Polarized Human Respiratory Epithelial Cells

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CD23-Dependent Transcytosis of IgE and Immune Complex across the Polarized Human Respiratory Epithelial Cells

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IgE-mediated allergic inflammation occurs when allergens cross-link IgE on the surface of immune cells, thereby triggering the release of inflammatory mediators as well as enhancing Ag presentations. IgE is frequently present in airway secretions, and its level can be enhanced in human patients with allergic rhinitis and bronchial asthma. However, it remains completely unknown how IgE appears in the airway secretions. In this study, we show that CD23 (FcεRII) is constitutively expressed in established or primary human airway epithelial cells, and its expression is significantly upregulated when airway epithelial cells were subjected to IL-4 stimulation. In a transcytosis assay, human IgE or IgE-derived immune complex (IC) was transported across a polarized Calu-3 monolayer. Exposure of the Calu-3 monolayer to IL-4 stimulation also enhanced the transcytosis of either human IgE or the IC. A CD23-specific Ab or soluble CD23 significantly reduced the efficiency of IgE or IC transcytosis, suggesting a specific receptor-mediated transport by CD23. Transcytosis of both IgE and the IC was further verified in primary human airway epithelial cell monolayers. Furthermore, the transcytosed Ag–IgE complexes were competent in inducing degranulation of the cultured human mast cells. Because airway epithelial cells are the first cell layer to come into contact with inhaled allergens, our study implies CD23-mediated IgE transcytosis in human airway epithelial cells may play a critical role in initiating and contributing to the perpetuation of airway allergic inflammation. The Journal of Immunology, 2011, 186: 000–000.

Allergic diseases are the most common type of all immunologically mediated disorders affecting 20–30% of the U.S. population and are increasing in incidence in the developed world (1). Airway allergy is one type of allergic disorder that affects both adults and children. Typically, the allergic inflammation in the airway is characterized by a prominent increase in the numbers of immune cells, such as eosinophils, and an imbalance between the Th1 and Th2 cell-mediated immune response with a shift toward Th2 cytokine release, such as IL-4, IL-5, and IL-13, and the considerable increase of allergen-specific IgE (2–4). IL-4 and IL-13 can further promote B cell class switch response with a shift toward Th2 cytokine release, such as IL-4, IL-5, and IL-13, and the considerable increase of allergen-specific IgE (2–4). IL-4 and IL-13 can further promote B cell class switch to increase IgE production (4–6). IgE is considered a major player in airway allergic inflammation. Clinical studies have found a close association between asthma and serum IgE levels, and the amount of allergen-specific IgE can be dramatically increased in sensitized or atopic patients (7, 8). Allergen-specific IgE is located in the human airway, such as in nasal mucosa from allergic patients who suffer allergic rhinitis in sinonasal tissue, nasal polyposis, and allergic fungal rhinosinusitis (9–12). This observation is further supported by the fact that B cells from the nasal mucosa of allergic individuals locally synthesize IgE (13, 14). As a result, allergen-specific IgE consistently appears in airway fluids in asthmatic patients obtained with or without bronchial provocation with the allergen. The proposed role of IgE in the initiation of the asthmatic allergy is that the allergy is triggered by Ags cross-linking IgE bound to IgE receptors on the surface of mast cells. This, in turn, triggers degranulation of mast cells, which releases potent inflammatory mediators (1, 4). In the airway mucosa, these inflammatory mediators, including histamine, lipid mediators, proteases, and so on, can act on nearby cells, such as epithelial cells and smooth muscle cells and further cause airway obstruction. However, for inhaled allergens to gain access to immune effector cells in the lamina propria, they must first cross the respiratory epithelium containing ciliated columnar, mucous-secreting goblet, and surfactant-secreting Clara cells. These cells can form a highly regulated and impermeable barrier made possible through the formation of tight junctions localized in the apical part of the columnar cells. In general, tight junctions prevent the free uptake and passage of macromolecules such as IgE and allergens. Hence, the exact mechanisms responsible for the cross-talk between allergen/IgE and immune effector cells in the airway remains poorly understood.

Two receptors for IgE, high-affinity receptor FceRI and low-affinity receptor FcεRII (CD23), play important roles in the pathogenesis of airway allergic inflammation. FceRI present in airway mast cells is sensitized by the allergen-specific IgE upon exposure to a specific allergen. FcεRI is activated to signal for the production of potent mediators that are responsible for clinical symptoms in allergic diseases (15). In humans, CD23 has two isoforms—CD23a and CD23b. The amino acid sequences of the CD23a and CD23b proteins differ only in their six of seven N-terminal residues, a region that corresponds to the cytoplasmic domain (16, 17), suggesting that this region regulates divergent intracellular trafficking and/or signaling pathways. Unlike FceRI,
both CD23 isoforms exhibit a type II membrane glycoprotein structure with a C-terminal C-type lectin head that binds IgE in a calcium-independent manner (18). Although CD23 monomers display lower-affinity binding for IgE, the membrane-bound CD23 can also form trimers, which enables CD23 to bind IgE with higher affinity (4, 17, 19). More interestingly, the stalk region of CD23 is susceptible to proteolysis by enzymes, such as the metalloproteinase ADAM10, to release various soluble CD23 (sCD23) fragments (20–22). sCD23 also has IgE-binding activity (23), and its level is increased in allergies (24). CD23 is constitutively and inducibly expressed in variety of cell types, including B cells, eosinophils, monocytes, and Langerhans cells (16, 18, 25, 26). More interestingly, CD23 has various functions, such as mediating B cell growth, enhancing IgE-mediated Ag presentation (27, 28), and regulating IgE homeostasis (29–32).

The functional significance of IgE found in the mucosal secretions of the human airway remains elusive. Several elegant studies have demonstrated a potential function for CD23 to transport IgE and IgE-derived immune complex (IC) across the polarized human intestinal epithelial monolayer (33–35). Furthermore, the facilitated transport of IgE and uptake of Ags by CD23 are essential steps in the initiation of rapid allergic inflammation in the intestine in a murine model (36, 37). All these experiments suggest a role of CD23-mediated IgE transport in food allergies. However, it remains completely unknown whether a similar mechanism of transport of IgE or IC exists in human airway epithelium. If such a function exists, allergen-specific IgE in the airway may capture airborne allergens and form IC in human airway tract. Subsequently, because of the especially sensitive environment of the airway tract, these IC may function as more potent inducers or triggers of immune responses in the airway and thus contribute to the perpetuation of airway inflammatory responses. As the first step, we found human CD23 was constitutively expressed in a variety of epithelial cells derived from human airway. Most importantly, CD23 was capable of transporting human IgE or IgE-derived IC across polarized human airway epithelial monolayers. By mimicking the allergic conditions, we also demonstrated that epithelial cell exposure with IL-4 resulted in the upregulation of CD23 expression accompanied by enhanced transepithelial transport of IgE and, more importantly, of IgE-derived IC. These transported IC were then capable of inducing the degranulation of human mast cells. Our findings may provide important evidence that CD23 in human airway epithelial cells is very likely to have a pivotal role in the initiation and development of airway allergic inflammation.

**Materials and Methods**

**Abs, cells, and reagents**

The human airway epithelial cell lines A549 and Calu-3, intestinal epithelial cell line HT-29, and monocytic cell line U937 were purchased from the American Type Culture Collection (Manassas, VA). The human nasal epithelial cell line New 562 was a gift from Dr. J. R. Zhang (Albert Einstein College Medical School, Albany, NY); human bronchial epithelial cell line 16HBE14o- was from Dr. D. Gruenert (University of California, San Francisco, CA). Human B cell line RPMI 8866 was from Dr. L. Bridges (University of California); human CD23 Ab-secreting murine hybridoma EBVCS1 (38, 39) was a gift from Dr. B. Sugden (University of Wisconsin, Madison, WI). Human IgE was purchased from The Scripps Laboratory (La Jolla, CA), and human IgE-containing κ L chains were from Abbiotec (San Diego, CA). Goat anti-human IgE and mouse IgG1 were purchased from Sigma-Aldrich (St. Louis, MO). Ab against human IL-4, rIL-13, chimeric anti–NP-IgE, chimeric anti–NP-IgG, and mouse anti-human CD23 Ab clone Tu1 were obtained from AbD Serotec (Raleigh, NC). JW8 cells secreting the NP-specific human IgE were obtained from Dr. H. Gould (Kings College, London, U.K.). Mouse anti-human IgE and mouse anti-human trypsin-specific Ab were from Abcam (Cambridge, MA). rIL-4 and sCD23 were purchased from R&D Systems (Minneapolis, MN). Proteinase inhibitor mixture was obtained from Calbiochem (San Diego, CA). The CD23HA plasmid was received from Dr. Z. Werb (University of California). Ab IDEC-152 was a gift from Dr. M. Kehry (Biogen Idec, San Diego, CA).

**RT-PCR**

Semiquantitative RT-PCR was performed as described previously (40). In brief, total RNA was isolated from stimulated human IL-4 (20 ng/ml) and mock-stimulated cells (2 × 106/ml) in TRIzol reagents (Invitrogen), according to the manufacturer’s instructions. RT-PCR was performed using a one-step RT-PCR kit (Qiagen). Negative control was set without an RNA template. Primers for amplification of CD23a and CD23b (34) and GAPDH (40) have been described previously. For the amplification of CD23 mRNA, 150 ng total RNA was used. For IL-4 stimulation, 5 ng total RNA from U937 and 40 ng total RNA from A549 or Calu-3 were used for amplification. Thirty-six cycles of PCR amplification were performed in a 20-μl volume. Each cycle consisted of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 20 s. An additional 10 min was applied for the final extension. PCR products were resolved on 1.5% agarose gels and visualized by ethidium bromide staining. Integrated density values for CD23 band intensities were analyzed by densitometry using Adobe Photoshop and normalized to the GAPDH values to yield a semiquantitative assessment.

**Gel electrophoresis and Western blotting**

Gel electrophoresis and Western blot were performed as described previously (40). Protein concentrations were determined by the Bradford method. The cell lysates were subjected to 12% SDS-PAGE gel electrophoresis under reducing conditions. Proteins were electrotransferred onto a nitrocellulose membrane (Schleicher & Schuell Microscience). The membrane was blocked with 5% skim milk powder in PBS. The membranes were probed with anti-CD23 mAb EBVCS1 for 1 h or overnight, followed by incubation with HRP-conjugated rabbit anti-mouse Ab (Pierce). For detecting human IgE, membranes were probed with HRP-conjugated goat anti-human IgE Ab. For detecting NP Ag in NP-BSA, membranes were first incubated with chimeric anti–NP-IgG, followed by HRP-conjugated rabbit anti-human IgG Fc. All blocking, incubation, and washing were performed in PBST solution (PBS and 0.05% Tween 20). Proteins were visualized by an ECL method (Pierce).

**Flow cytometry**

Surface and intracellular expression of human CD23 was examined in either fixed or permeabilized A549 or RPMI 8866 cells by flow cytometry. For staining, 1 × 106 cells were washed with FACS washing buffer (2% FBS in PBS) and followed by blocking with PBS containing 3% normal goat serum (Rockland, Gilbertsville, PA)/PBS on ice for 20 min. For surface CD23 staining, the cell suspensions were incubated with anti-CD23 EBVCS1 or isotype-matched mouse IgG1 (1 μg/ml) for 30 min, washed, and further incubated with Alexa Fluor 488-conjugated goat anti mouse (1:500) Ab at 4°C. Surface staining was carried out at 4°C to minimize internalization. For intracellular staining, the cells were first permeabilized and fixed with Cytofix/Cytoperm (BD Pharmingen) on ice for 20 min, then washed with washing/permeabilization buffer and blocked with 3% normal goat serum. The cells were further incubated with EBVCS1 Ab (1 μg/ml) diluted in washing/permeabilization buffer, washed, and incubated with Alexa Fluor 488-conjugated goat anti-mouse (1:500) Ab. After washing cells, they were analyzed using a FACScAria II and the software FlowJo.

**Immunocytochemistry**

Immunocytochemistry was performed as described previously (41). The epithelial cells were grown on coverslips to 70–80% confluence overnight.
or in Transwell inserts to allow polarization. The cells were rinsed in cold PBS and cold-fixed in 4% paraformaldehyde in PBS for 20 min at 4 °C. The cells were washed and quenched with 100 mM glycine for 10 min. After washing, the cells were permeabilized for 30 min with 0.1% Triton X-100 and blocked with 3% normal goat serum. Cells were incubated with affinity-purified anti-CD23 EBVCS1 Ab (1 µg/ml) in PBST (0.05% Tween 20/PBS) or mouse anti-human IL-4R Ab with 3% normal goat serum in PBS for 1 h and further incubated with Alexa Fluor 488-conjugated goat anti-mouse Ab (1:500). The cells grown in the Transwell were stained using procedures above with the addition of Abs on both sides. Finally, membrane was cut from the Transwell and mounted on the slide and analyzed. Negative control was performed by incubating the isotype-matched normal mouse IgGl Ab. Cell nuclei were counterstained with DAPI (0.5 g/ml; Molecular Probes). In PBS, each step, cells were washed with 0.1% Tween 20 in PBS. The ProLong antifade kit was used to mount coverslips (Molecular Probes). The images were taken using a Zeiss LSM510 laser scanning confocal microscope (Zeiss Microimaging).

**ELISA**

The IgE concentration was measured with ELISA. ELISA plates (Nunc) were coated with rabbit anti-human IgE Ab (10 µg/ml) overnight at 4 °C. Plates were then washed three times with PBST (0.05% Tween 20) and blocked with 10% FBS in PBS for 1 h at room temperature. The transcytosed samples or IgE standards diluted in 10% FBS in PBS were incubated for 2 h at room temperature. Mouse anti-human IgE (1:10,000; Abcam) and HRP-conjugated rabbit anti-mouse Ab (1:10,000; Pierce) were used for detection of human IgE. For detection of chimeric IgE, HRP-conjugated goat anti-human IgE (1:10,000; Sigma-Aldrich) was used. A colorimetric assay was done with tetramethylbenzidine and hydrogen peroxide and a Victor III microplate reader (PerkinElmer).

**Transcytosis assay**

Transcytosis was performed as previously described with modifications (34, 40, 42). Calu-3 cells (1 × 10^5/ml) were grown onto 0.4-mµm-pore size Transwell inserts (BD Biosciences) to form a monolayer exhibiting transepithelial electrical resistances (TER; 450–900 Ω/cm²). TER was measured using a tissue-resistance measurement equipped with planar electrodes (World Precision Instruments, Sarasota, FL). Monolayers were equilibrated in serum-free DMEM or stimulated with human IL-4 (20 ng/ml) and concentrated using Ultracel YM-10 (Millipore, Billerica, MA).

**Results**

**Human respiratory epithelial cells express CD23**

Epithelial cell lines from human nasal, trachea, and lung were used in studies on human CD23 expression. These cell lines maintain the differentiation characteristics of their tissues of origin with proper polarity. Human CD23 is expressed in two isoforms: CD23a and CD23b (34). Results from RT-PCR amplification showed that all airway epithelial cells expressed the CD23b (Fig. 1A), but not the CD23a (Fig. 1B), transcript. Mononuclear cell line U937 was used as a positive control. The PCR products were sequenced to verify CD23b isoform-specific transcripts. To verify CD23 expression at the protein level, we blotted the cell lysates from Detroit 562, Calu-3, and A549 cells with EBVCS1 mAb, a specific IgG1 Ab for human CD23 (38, 39). These airway epithelial cells expressed protein bands identical to 293T and intestinal T84 cells transfected with vectors expressing human CD23b cDNA in a Western blot (Fig. 1C, left panel). Two bands were identified; however, the top band (50 kDa) was also expressed in cell lysates from Chinese hamster ovary cell lysates (Fig. 1C, right panel), suggesting the lower band (45 kDa) is CD23 specific. In addition, immunofluorescence staining of Calu-3 cells by EBVCS1 mAb (Fig. 1D, left panel), but not for isotype-matched IgGl Ab (Fig. 1D, right panel), revealed the expression of CD23 protein.

CD23 is known to be expressed intracellularly and/or at the cell surface (44). To show the expression pattern of CD23 in airway epithelial cells, we stained lung epithelial cells A549 with EBVCS1 mAb for cell surface or intracellular expression by flow cytometry (Fig. 1E). The specificity and expression pattern of CD23 was, in parallel, performed in a RPMI 8866 human B cell line. In contrast, to ~84% human B cell line, only ~36% A549 cells expressed CD23 at the cell surface. However, permeabilization of A549 cells revealed substantial increases of CD23 positive cells from ~36 to 99%. The distinct cellular distribution of CD23 between airway epithelial and B lymphocytes has not been appreciated in previous studies. In addition, activation of human eosinophils leads to increased surface expression of CD23 (44). Our data showed that IL-4 stimulation also increased the surface expression of CD23 in A549 cells (data not shown). Therefore, we conclude that CD23 is mainly expressed intracellularly in lung epithelial cell A549, suggesting CD23 may function as an intracellular IgE receptor in airway epithelial cells. IL-4 stimulation upregulates the expression of CD23 in human airway epithelial cells

The Th2-type T cells mediate airway allergic inflammation by producing IL-4 and IL-13. IL-4 and IL-13 can enhance the expression of CD23 in B cells, monocytes, and intestinal epithelial cells (45, 46). The results showed that, when airway epithelial cells A549 and Calu-3 cells were subjected to IL-4 (20 ng/ml) stimulation for 24 h, CD23b mRNA expression, which was measured by semiquantitative RT-PCR, was significantly enhanced (Fig. 2A, lanes 5, 7). In contrast, CD23a mRNA expression was measured by adding the substrate 1 mM 3,3′-diaminobenzidine dissolved in 50 mM Tris HCl (pH 7.6), 120 mM NaCl, and 20 µg/ml heparin solutions and incubated for 1 h at 37 °C. To quantify the substrate cleavage, the absorbance at 405 nm was measured using a microplate reader. The results were read out at 405 nm. In addition, tryptase in LAD2 mast cells was analyzed by immunofluorescence staining with human tryptase-specific Ab in a confocal microscope.
not induced by 24-h IL-4 stimulation in A549 and Calu-3 cells (Fig. 2B, lanes 5, 7). As expected, mRNA expression of both CD23a and CD23b isoforms in U937 were significantly enhanced after IL-4 stimulation. Furthermore, as shown in Fig. 2C, expression of IL-4R was shown by immunofluorescence staining with IL-4R mAb but not with isotype-matched IgG Ab when Calu-3 cells were grown in Transwell inserts and polarized.

**CD23 transports human IgE bidirectionally across the polarized Calu-3 epithelial cells**

The human airway epithelial cell Calu-3 was polarized and used to transcytose IgG bidirectionally when grown on both sides of filter supports (40). Hence, we used Calu-3 as a model cell line to examine transcytosis of human IgE. First, CD23 expression was verified by immunofluorescence staining with EBVCS1 mAb and the protein bands were visualized by the ECL method. The arrow indicates the location of the CD23. D, Immunofluorescence staining of Calu-3 cell line. The Calu-3 cells were grown on glass coverslips, fixed with 4% paraformaldehyde, and permeabilized in 0.1% Triton X-100. Subsequently, the cells were incubated with goat serum for blocking and affinity-purified mouse anti-CD23 EBVCS1 mAb, followed by staining with an Alexa Fluor 488-conjugated goat anti-mouse Ab. The Calu-3 cell was stained with an isotype-matched mouse IgG1 Ab as negative control (right panel). The nucleus was stained with DAPI and photographed through a fluorescence microscope. Samples were viewed using the same contrast and brightness settings (original magnification ×630).

**FIGURE 1.** Human airway epithelial cell lines express CD23. A and B, CD23b, but not CD23a, isoform was expressed in human airway epithelial cells. Total RNA was extracted by TRIZol reagent from airway epithelial cells Detroit 562 (lane 4), 16HBE14o- (lane 5), Calu-3 (lane 6), and A549 (lane 7). Monocyte cell line U937 (lane 2) and intestinal epithelial cell HT-29 (lane 3) were used as a positive control. RT-PCR was performed by using CD23a or CD23b isoform-specific primers as described in Materials and Methods. Amplified PCR products, 170 bp for CD23a and 131 bp for CD23b, were electrophoresed in 1.5% agarose gel and stained with ethidium bromide. NC represents a negative control without template in RT-PCR. Similar PCR products amplified with a GAPDH-specific primer pair were used as internal controls. The arrow indicates the location of the amplification products for CD23 and GAPDH. The m.w. in base pair is indicated on the left. C, CD23 protein was expressed by human airway epithelial cells. The cell lysates (60 μg) from 293T-CD23bHA (lanes 1, 7) and T84-CD23b (lane 2), Detroit 562 (lane 3), Calu-3 (lane 4), A549 (lane 5), and Chinese hamster ovary (CHO) (lane 6) were separated by electrophoresis on 12% SDS-PAGE gel under reducing conditions. The proteins were transferred onto nitrocellulose membrane and blotted with mouse anti-human CD23 EBVCS1 mAb. The blots were incubated with HRP-conjugated rabbit anti-mouse IgG, and the protein bands were visualized by the ECL method. The arrow indicates the location of the CD23. D, Immunofluorescence staining of Calu-3 cell line. The Calu-3 cells were grown on glass coverslips, fixed with 4% paraformaldehyde, and permeabilized in 0.1% Triton X-100. Subsequently, the cells were incubated with goat serum for blocking and affinity-purified mouse anti-CD23 EBVCS1 mAb, followed by staining with an Alexa Fluor 488-conjugated goat anti-mouse Ab. The Calu-3 cell was stained with an isotype-matched mouse IgG1 Ab as negative control (right panel). The nucleus was stained with DAPI and photographed through a fluorescence microscope. Samples were viewed using the same contrast and brightness settings (original magnification ×630). E, Cellular distribution of human CD23 expression patterns in lung epithelial cell and B lymphocytes. Cell surface and intracellular expression patterns of CD23 in either fixed or permeabilized lung epithelial cell A549 and B lymphocyte RPMI 8866 were measured by flow cytometry analysis. Cells were stained as described in the Materials and Methods. Results are expressed as histograms of fluorescence intensity (log scale). The histograms represent staining of cells with anti-CD23 EBVCS1 mAb or isotype-matched mouse IgG1 and then with Alexa Fluor 488-conjugated goat anti-mouse IgG Ab. Values in each rectangle correspond to the proportion of cells stained with the anti-CD23 Ab relative to the control Ab. The staining for A549 and RPMI 8866 was conducted three times with similar results.
CD23 expression was upregulated in human airway epithelial cells following exposure to IL-4 treatment. A and B, Human airway epithelial cells Calu-3 and A549 were incubated with or without IL-4 (20 ng/ml) for 24 h. Monocytic cell line U937 was used as a positive control. At the end of incubation period, the cells were washed, and total RNA was extracted by TRIzol reagent. Total RNA was subjected to semiquantitative one-step RT-PCR with CD23b (A) or CD23a (B)-specific primers. GAPDH was used as internal control. PCR products were run in 1.5% agarose gels and stained with ethidium bromide. Densitometry analysis of CD23b (A, right panel) or CD23a (B, right panel) band intensities, which were normalized to GAPDH, was presented. NC: a negative control without template RNA in RT-PCR. *p < 0.05, **p < 0.01. C, Confocal microscopy detection of IL-4R. Calu-3 cells (1 × 10^5/ml) were grown on 0.4-μm-pore size inserts. Calu-3 cells were polarized, fixed, and permeabilized in 0.1% Triton X-100. IL-4R was detected by immunostaining with Alexa Flour 488 (green). Cells were stained with ZO-1 (red) to show the level of the tight junction and DAPI to stain nuclei (blue). The XY sections are taken at the level of the ZO-1 staining, and the XZ sections are shown at the bottom. The red staining indicates the tight junctions at the apical pole of the Calu-3 cells (original magnification ×630).

3C, lane 3) or the basolateral (Fig. 3C, lane 4) chamber was transported in the opposite directions across the Calu-3 monolayer as assessed by blotting of IgE H chain. Transport of IgE was not detected in monolayers incubated at 4˚C (Fig. 3C, lane 2), ruling out the leaking possibility of IgE Ab across Calu-3 monolayers. Because CD23 was able to transport IgE bidirectionally across the polarized epithelial cells, we also used protein L beads that can specifically bind to human CD23 in Calu-3 cells isotype-controlled transcytosis. To exclude this possibility, we performed RT-PCR analysis for the expression of the α-chain of FcεRI. Our results showed that airway epithelial cells failed to express FcεRI (data not shown) at 35 cycles of PCR-amplifying condition. To show whether CD23 transcytosis is dependent on the expression of CD23 in Calu-3 cells, two experiments were performed. First, IgE incubation with sCD23 significantly inhibited the amount of IgE transcytosed (Fig. 3E). Second, either apically or basolaterally applied CD23-specific Ab IDEC-152 (1 μg/ml) significantly inhibited the transcytosis of IgE from the apical to the basolateral (Fig. 3F) or vice versa (data not shown). IDEC-152 mAb is anti-human CD23 mAb (lgG1) consisting of primate (Cynomolgus macaque) variable regions and human constant regions, and for this reason, we did not include an isotype-matched IgG1 mAb as a control. IDEC-152 has been shown to block synthesis of IgE from human B cells in vitro (47). The protein concentrations for block were previously determined in a serial dilution. Both experiments demonstrated the specificity of CD23-mediated IgE transport in Calu-3 cells. We further defined the role of endosomal trafficking in CD23-mediated IgE transcytosis. Calu-3 cell inserts were incubated with or without bafilomycin A1 (0.1 μmol), which interferes with the intracellular trafficking by inhibiting endosome acidification as shown in transcytosis of IgE or IgG in intestinal epithelial cells (34, 42). As shown in Fig. 3G, bafilomycin significantly inhibited apical to basolateral IgG transcytosis, although it was unable to completely block CD23-mediated IgE transcytosis. As an internal control, the bafilomycin completely blocked IgG transcytosis across Calu-3 monolayer, which is in agreement with the previous finding (Fig. 3H) (42). Hence, we conclude that IgE entering both apically and basolaterally directed transcytotic pathways in lung epithelial cells is dependent on CD23 expression.

CD23 transcytoses IgE-derived IC across polarized Calu-3 cells

Previous studies have shown that IgE is frequently present in the mucosal secretions of airway tract, and its level can be enhanced in human patients with asthma (8, 11). Transferring of the intact allergen molecule across the airway epithelial barrier may be an important event in priming a host for an allergen, and subsequent exposure of same allergen leads to the development of allergic inflammation. CD23-mediated IgE transport across the polarized Calu-3 epithelial cells suggests allergen-specific IgE can also capture airborne allergen and form IC in the lumen of the airway tract. Therefore, we further examined whether CD23 could facilitate transepithelial transcytosis of IgE-derived IC across Calu-3 monolayers. We used chimeric IgE that consists of mouse Fab directed against NP molecules and human Fc fragment. Chimeric IgE can bind well to human CD23 as demonstrated previously (34). IC was formed by incubating the chimeric IgE with NP-BSA at 37˚C. Transcytosis of IC across polarized Calu-3 epithelial cells was examined from the apical (luminal) to basolateral direction to
mimic the in vivo condition where the airway epithelial cells are usually exposed to the inhaled Ags. As shown in Fig. 4A, the apically applied IgE–NP-BSA complex was transported after 2 h in the basolateral direction across Calu-3 monolayer at 37˚C incubation (Fig. 4A, lane 4), as assessed by blotting of NP Ag. IgE incubated at 4˚C (Fig. 4A, lane 2) or NP-BSA Ag alone (Fig. 4A, lane 3) was not transcytosed across polarized Calu-3 cells. The chimeric IgE in the IC was also detected at 37˚C, but not at 4˚C, by ELISA (Fig. 4B). Furthermore, the IC transcytosed across the Calu-3 monolayer were detectable within 30 min after transcytosis initiation (Fig. 4C, lane 3). The transcytosis of IC was also decreased when the apically applied IC were serially diluted (Fig. 4D, lanes 3, 4); however, nonlinearity was detected between the applied IC and the transcytosis efficiency in the assay.

To further show whether this transport is dependent on CD23, two experiments were performed. First, we examined the influence of sCD23 on transcytosis of NP-BSA Ags in the presence of IgE. The incubation of sCD23 with the IC before adding it to the Calu-3 cells was found to increase the transcytosis of IC (Fig. 4E, lane 2). In addition, IDEC-152 mAb (Fig. 4F, lane 2) inhibited the transcytosis of IC at 37˚C, but not at 4˚C, in the presence of sCD23. As shown in Fig. 4G and 4H, calcofluor white was used to detect the presence of basolaterally directed transport of IgE and IgG. The results were representative of three individual experiments. A, apical; B, basolateral.
monolayer significantly reduced the transcytosis of NP-BSA Ag in an sCD23 protein concentration (10–20 μg/ml)-dependent manner (Fig. 4E, lanes 4, 5) in comparison with untreated IC at 37°C (Fig. 4E, lane 3). This suggests that sCD23 renders the IC unavailable for membrane CD23 on the Calu-3 monolayer. Second, we determined that the transcytosis of the IC was significantly reduced by CD23-specific IgG Ab IDEC-152 (Fig. 4F, lanes 3, 4). In a parallel experiment, IgE in IC was also reduced significantly (Fig. 4G).

**FIGURE 4.** Transepithelial transport of IC in Calu-3 cells. Calu-3 cells (1 × 10⁵/ml) were grown on 0.4-μm-pore size Transwell filters. The cells were allowed to get polarized, and the transcytosis was performed when the TER value reached at least 450–900 Ωcm². The IC was formed by incubating chimeric anti-NP IgE (20 μg/ml) with NP-BSA (10 μg/ml) at 37°C for 45 min. A, apical; B, basolateral; STD, standard. A and B, Transcytosis of IC in the Calu-3 cell line. The NP-BSA Ag alone (lane 3) or IgE-NP-BSA IC (lanes 2, 4) was added to the apical chamber for incubation at 4°C (lane 2) or 37°C (lanes 3, 4) for 2 h. The media from the basolateral chamber were collected and concentrated for detecting NP-BSA Ag in a Western blot (A) or total IgE in an ELISA (B). C and D, Effect of incubation time or concentration of the IC on the transcytosis. IC was added to the apical chamber at different times (C) or serially diluted with normal medium (D) and then incubated at 4°C (lane 2) or 37°C (C, lanes 3–5) or 37°C (D, lanes 3, 4) to allow transcytosis as indicated. The medium from the basolateral chamber was collected, concentrated, and blotted for detection of NP-BSA Ag. E, Effect of sCD23 on the transcytosis of IC. The IC was preincubated with sCD23 at the indicated concentration before addition to the apical chamber. Then, the IC were incubated at 4°C (lane 2) or 37°C (lanes 3–6) for 2 h to allow transcytosis. Supernatant from the basolateral chamber was sampled and blotted for NP Ag (E). The graph (E, bottom panel) represents the densitometry analysis of NP-BSA bands obtained from the transcytosed IC treated with (lanes 4–6) or without (lane 3) sCD23 proteins. Results were representative of three individual experiments. *p < 0.05, **p < 0.01. F and G, Effect of CD23-specific Ab on the transcytosis of IC. CD23-specific IDEC-152 mAb at indicated concentration was incubated with Calu-3 cells at 4°C for 1 h before the addition of IC to the apical chamber. Then, the IC were incubated at 4°C (lane 2) or 37°C (lanes 3–5) for 2 h to allow the IC transcytosis. Supernatant from the basolateral chamber was sampled and measured for NP Ag in a Western blot (F) or total IgE in an ELISA (G). The graph (F, bottom panel) represents the densitometry analysis of NP-BSA bands obtained from the transcytosed IC with samples treated with (F, lanes 3 and 4) or without (F, lane 5) IDEC-152 Ab. Results were representative of three individual experiments. *p < 0.05.
**IL-4 enhances the transcytosis of IgE or IC in polarized Calu-3 cells**

IL-4 treatment enhanced CD23b expression in the airway epithelial cells (Fig. 2). We further examined whether IL-4 treatment stimulates the transcytosis of either IgE or IC across the Calu-3 epithelial cells. To show this effect, the polarized Calu-3 monolayers were treated with or without IL-4 (20 ng/ml) exposure before either IgE or IC was added. As shown in Fig. 5A and 5B, the transcytosis of human IgE was significantly enhanced in both apical to basolateral (Fig. 5A) and basolateral to apical (Fig. 5B) directions with IL-4 exposure at 37°C in comparison with that of mock-treated cells. Kinetics of IgE transcytosis from the apical to basolateral direction in Calu-3 monolayer were shown in Fig. 5C. In a similar way, the transcytosis of IgE IC was also significantly enhanced in the presence of IL-4 stimulation at 37°C in comparison with that of mock-treated cells (Fig. 5D). The amount of IgE IC transported also exhibited a time course-dependent manner (Fig. 5E). In addition, the IC was barely detectable when Calu-3 monolayers were incubated at 4°C (Fig. 5D, lane 2), suggesting Calu-3 cells under 24-h exposure of IL-4 stimulation did not significantly alter the transepithelial resistance. Taken together, IL-4 treatment increased the transcytosis of both IgE and IC across Calu-3 cells.

**FIGURE 5.** Effect of IL-4 treatment on transcytosis of IgE and IC in the polarized Calu-3 cells. Calu-3 cells (1 × 10⁵/ml) were grown on 0.4-μm-pore size Transwell filters. The cells were allowed to get polarized, and the transcytosis was performed when the TER value reached at least 450–900 Ω/cm². Calu-3 cells were treated with or without IL-4 (20 ng/ml) for 24 h. A, apical; B, basolateral. A and B, Human IgE (0.5 μmol/l) was added into the apical (A) or basolateral (B) chamber to allow transcytosis for 2 h at 4°C (lane 2) and 37°C with or without IL-4 treatment (lanes 3, 4). Supernatant from the apical chamber was sampled, concentrated, and blotted for IgE. The relative integrated band intensities of the IgE transcytosed across the Calu-3 monolayer from the top panel were calculated by densitometry analysis (bottom panels). C, Kinetics of IgE transcytosis in lung Calu-3 monolayer. Human IgE was added to the apical chamber of polarized Calu-3 epithelial cells that were pretreated with (○) or without IL-4 (●) for 24 h and incubated at 37°C for an additional 2 h at indicated time points. The medium from the basolateral chamber was collected and concentrated for measuring total IgE by ELISA. D and E, IgE-derived IC was added into the apical chamber to allow transcytosis for 2 h at 4°C (lane 2) and 37°C in the presence or absence of IL-4 treatment (D, lanes 3, 4). E, Calu-3 monolayers were treated with IL-4 at indicated times (E, lanes 3–5). Supernatant from the basolateral chamber was sampled and blotted for NP-BSA Ag. The relative integrated band intensities of the IC transcytosed across the Calu-3 monolayer from the top panel were analyzed by densitometry (bottom panels). The image in E was processed by Adobe Photoshop.
Primary human tracheal and bronchial epithelial cells can transcytose both IgE and IC

To further verify the transcytosis results of either IgE or IC in human Calu-3 cell lines, we used commercially available primary human tracheal–bronchial epithelial tissues that were originally obtained from a human donor. Human tracheal/bronchial cell cultures (EpAirway tissues, AIR-100-SNAP) closely resemble in vivo conditions of human tracheal/bronchial cells. As the first step, CD23b, but not CD23a, expressions were verified by RT-PCR amplification with CD23-specific primers (Fig. 6A), Western blot (Fig. 6B), and immunofluorescence staining (Fig. 6C, left panel) with EBVCS1 mAb in these primary human epithelial cells. The specificity of the staining was confirmed with isotype-matched IgG control (Fig. 6C, right panel). CD23 expression was upregulated in the primary human tracheal bronchial epithelial cells in response to IL-4 treatment (data not shown). When human IgE was loaded onto either apical or basolateral chambers, these human primary tracheal–bronchial epithelial tissues were capable of transcytosing IgE from either the apical to basolateral or the basolateral to apical directions (Fig. 6D, 6E). IgE Ab failed to be detected in both directions when tissues were incubated at 4˚C. In addition, chicken IgY, which closely resembles human IgE in structure (48), was not transcytosed in both directions (Fig. 6D, 6E), demonstrating that the human IgE detected in transcytosis assay was the specific transport but not passively diffused through paracellular pathway or because of leakiness in model tissue. To further show whether the primary human tracheal and bronchial epithelial cells can also transcytose IC, we incubated these cells with IgE-derived IC in the apical chamber for 2 h. As shown in Fig. 6F, Ag–IgE complexes were transcytosed from the apical-to-basal at 37˚C, but not at 4˚C, as detected by Western blotting of NP-BSA Ags. Ag NP-BSA alone was undetectable in transcytosis by primary human tracheal and bronchial epithelial cells, further demonstrating the specificity of IgE-CD23–mediated transcytosis. Overall, all these evidences strongly indicate that IgE or IC can

![Image](98x227 to 486x521)

**FIGURE 6.** The primary human tracheal bronchial epithelial tissue transcytoses IgE and IC. All protein samples were separated on 12% SDS-PAGE gel under reducing condition, and proteins transferred to nitrocellulose membrane, blotted with protein specific Ab, and followed by HRP-conjugated secondary Ab. The blot was visualized by ECL method. A, The primary normal human tracheal bronchial epithelial cells (TBEC; AIR-100) expressed CD23b but not CD23a mRNA. Total RNA was extracted by TRizol reagent from primary epithelial cells that were grown in Transwell inserts consisting of TBEC forming highly differentiated and pseudostratiﬁed polarized epithelial tissue. The purified RNA was subjected to one-step RT-PCR with CD23-specific primers. The PCR products were electrophoresed on 1.5% agarose gel and DNA stained and identiﬁed with ethidium bromide. B, CD23 expression was upregulated in the primary human tracheal bronchial epithelial cells in response to IL-4 treatment (data not shown). When human IgE was loaded onto either apical or basolateral chambers, these primary human tracheal–bronchial epithelial tissues were capable of transcytosing IgE from either the apical to basolateral or the basolateral to apical directions (Fig. 6D, 6E). IgE Ab failed to be detected in both directions when tissues were incubated at 4˚C. In addition, chicken IgY, which closely resembles human IgE in structure (48), was not transcytosed in both directions (Fig. 6D, 6E), demonstrating that the human IgE detected in transcytosis assay was the specific transport but not passively diffused through paracellular pathway or because of leakiness in model tissue. To further show whether the primary human tracheal and bronchial epithelial cells can also transcytose IC, we incubated these cells with IgE-derived IC in the apical chamber for 2 h. As shown in Fig. 6F, Ag–IgE complexes were transcytosed from the apical-to-basal at 37˚C, but not at 4˚C, as detected by Western blotting of NP-BSA Ags. Ag NP-BSA alone was undetectable in transcytosis by primary human tracheal and bronchial epithelial cells, further demonstrating the specificity of IgE-CD23–mediated transcytosis. Overall, all these evidences strongly indicate that IgE or IC can...
enter the transcytotic pathways in primary human airway epithelial cells.

**CD23-mediated transcytosis of IC induces human mast cell degranulation**

IgE-mediated hypersensitivity reactions are mediated by Ag cross-linking of IgE Abs bound to its high-affinity receptor (FcεRI) on immune cells such as mast cells (4, 7). Because mast cells reside in close proximity to airway epithelial cells in the respiratory tract, we tested the ability of transcytosed Ag–IgE complexes to trigger the degranulation in human mast cells. To mimic the natural condition, we cultured LAD2 cells (1 × 10⁵/ml) in basolateral chambers when Calu-3 cells were grown in the Transwell inserts. The LAD2 is a human mast cell analog that can be stimulated to degranulate in an IgE-dependent manner (49). The supernatant from the basolateral chamber was obtained 3 h later after apical addition of the IC, IgE, and Ag alone. Although mast cell degranulation occurs rapidly, the supernatant sampled 3 h after addition of the IC ensured enough Ags to be transported in the experiment. The degranulation of LAD2 cells was assessed by both tryptase and β-hexosaminidase assays. As shown in Fig. 7A, as a positive control, direct incubation of LAD2 mast cells with IC induced high levels of mast cell degranulation. Also, LAD2 cells exhibited significant degranulation that was triggered by the transcytosed IC in comparison with that of IgE or Ag alone in either tryptase or β-hexosaminidase analyses. When LAD2 cells were primed with NP-specific IgE prior to addition of the transcytosed IC, the level of degranulation was significantly enhanced in comparison with that of LAD2 cells primed with Ag-non-specific IgE Ab (Fig. 7A). The degranulation was also demonstrated by the immunofluorescence staining of tryptase in LAD2 cells; the intensity of tryptase staining was greatly reduced in the presence of the transcytosed IC in comparison with IgE or Ag alone (Fig. 7B). However, it would be informative to use an Ag...
with multiple identical epitopes in degradation assay. Therefore, IgE released from the airway epithelial cells together with the Ag in an IC form is capable of activating effector mast cells.

Discussion

The early inflammatory response during allergic rhinitis and asthma is strongly associated with inhaled allergens cross-linking specific IgE on the surface of mast cells, leading to their activation and release of primary inflammatory mediators (1, 50). The late-phase reaction is characterized by recruitment, activation, and tissue infiltration of leukocyte populations, including lymphocytes, eosinophils, basophils, and neutrophils (1, 4). Ab–Ag interaction in the airway drives early granulocyte recruitment (51). Recently, basophils were also found to function as APC for an allergen-induced Th2 response (52). Therefore, one of the major roles of the airway epithelium is to act as a barrier to restrict the Ags and other potentially noxious materials from penetrating into the mucosal lamina propria of the airway. It has been known that some allergens with protease character penetrate mucosa or dendritic cells directly sample the inhaled Ags from the respiratory lumen (53). In this article, we found that CD23 is capable of transport of IgE and the IC across polarized human airway epithelial monolayer.

CD23 expression has been shown in many immune cells. In this study, we have systemically demonstrated that the constitutive expression of human CD23 in epithelial cell lines is derived from the nose, trachea, and lung (Fig. 1). We were not able to obtain respiratory biopsy samples and therefore examined a model tissue as a measure of airway CD23 expression. The association between CD23 and IgE was also demonstrated in a CD23-dependent IgE uptake assay in the human airway Calu-3 cells, proving that CD23 is structurally intact in this cell type. Therefore, our results support and extend the previous finding that CD23 is constitutively expressed in human intestinal epithelial cells (34, 36). We further found that human airway epithelial cells expressed CD23b, but not CD23a, mRNA (Fig. 1). The absence of CD23a protein expression by airway epithelial cells may indicate lack of constitutive expression, but it may also be due to the absence of a required growth factor in cell culture media. Our study does not rule out expression of CD23a in intact tissues in vivo. Clearly, in situ hybridization studies are necessary for conclusive evidence regarding CD23 isoforms expressed by specific cell types in vivo. The expression of isoform-specific CD23 mRNA in human intestinal epithelial cells remains controversial (33, 34). It is possible that cells grown in nonpolarized or polarized conditions might affect the expression of CD23 isoform in polarized epithelial cells (35). Interestingly, intracellular expression of CD23 in airway epithelial cells suggests CD23 functions as an intracellular receptor for IgE.

CD23 transports human IgE in a polarized airway epithelial monolayer. The function of CD23 has been most extensively studied on B cells, where it is involved in IgE-mediated Ag capture and facilitated presentation to T cells or regulation of IgE homeostasis (17, 26). There is a paucity of information on the link between engagement of CD23 on the airway epithelial cells and the functional outcome. In airway epithelial cells, the CD23 protein was visualized on both the apical and basolateral membrane, suggesting that IgE may be transported in both directions across the epithelium (Fig. 3A). In fact, we demonstrated that the human lung epithelial cell Calu-3 cell line transcytosed the exogenously applied IgE, which occurred in both apical-to-basal and basal-to-apical directions (Fig. 3C). Most importantly, human IgE was transported bidirectionally at concentrations representing at both physiological and allergy conditions (Fig. 3D). In our studies, IgE was clearly released after transcytosis, although we did not determine the relative quantity of free IgE against that which remains associated with its receptor. To confirm the specific transport, we conducted additional experiments using either sCD23- or CD23-specific Ab. We showed that either of them was able to significantly decrease IgE transport across the Calu-3 cell monolayer (Fig. 3). These results, taken together with the temperature sensitivity of IgE transport, provide evidence that the trans-epithelial transport of IgE is mediated by the CD23 expressed in the airway epithelial cells. The IgE transport in airway epithelial cells was partially sensitive to bafilomycin, suggesting that the transcytosis occurs also through endosomes. Both IgA and IgG transcytosis by the polymeric IgR and FcRn have also been shown to be sensitive to agents that interfere with pH gradients (42, 54). In contrast to FcRn-mediated IgG and polymeric IgR-mediated IgA transcytosis, it remains unclear how CD23 releases IgE at cell surface after transcytosis. Although the intracellular trafficking mechanism of CD23 has just begun to be explored, the similar ability of CD23 and FcRn to mediate bidirectional transcytosis suggests possible similarities in their transport mechanism.

CD23 transports IgE-derived IC across the airway epithelial barrier. The evidence of the airway epithelial cells transporting the IgE suggests that when a host inhales allergen, it may combine with the allergen-specific IgE present on the lumen or cell surface to form IC, and it can then be transcytosed across the airway epithelial barrier. To mimic the natural condition, we performed the transcytosis of IC from the apical to basolateral direction and found that Calu-3 monolayer efficiently transcytosed the IC but not Ag alone (Fig. 4A, 4B). In confirmation of the specificity of CD23 in transepithelial Ag transport, sCD23- or CD23-specific Ab blocked Ag uptake and transport of Ag in a concentration-dependent manner (Fig. 4). Furthermore, primary human tissues could also transcytose IgE or IC (Fig. 6). This is, however, not surprising given the fact that CD23 has been described previously as mediating endocytosis of IC in B cells (28, 55, 56). However, several issues should be noticed. First, in our experiment, an intact Ag was detected in the transport of the IC. This is consistent with the previous finding that CD23-mediated transepithelial transcytosis of the IC diverts Ag from lysosomal degradation in intestinal epithelial cells (34, 57). It is possible that by protecting Ag from degradation, CD23-mediated transcytosis of the IC may increase quantity of Ag delivered across the airway epithelium into the body. Second, the transport of the IC in the apical-to-basolateral direction was much more efficient than that of IgE alone, because we were able to easily detect the IC in our assay. The rationale for this discrepancy may be due to the sensitivity of detection methods for NP Ags. Alternatively, IC bound to CD23 in primary human tissues may cross-link CD23 molecule, initiate signaling cascade, and subsequently result in enhanced transcytosis of the IC. Studies in human intestinal epithelial cells have already found that the IC on the intestinal epithelial monolayers preferentially activate MAPK signaling pathway and subsequent release of chemokines IL-8 and CCL20 (58, 59). Although the exact mechanisms of this Ag transport system need to be further investigated, our results provide convincing evidence that human airway cell lines and primary airway tissue can transcytose IgE-derived IC, which is mediated by IgE and CD23. Therefore, CD23 can function as an Ag-sampling mechanism by transporting intact allergen–IgE complexes across human airway epithelia.

What might be the clinical implication and significance of CD23-mediated IgE and IC transport in human airway epithelial cells? Expression of CD23 and IgE level is enhanced in asthma patients (60, 61). CD23 is involved in regulating IgE synthesis in B cells (30), developing extrinsic allergic alveolitis in alveolar
transporter, which is regulated by IL-4. Both directions fit well with IgE across polarized airway epithelial monolayers. Taken together, a receptor involved in the binding and bidirectional transport of the IC on airway epithelial cells. Thereupon, our study may reveal a novel Ag transport or capture mechanism leading to an allergic response to inhaled allergens in an inflammatory and adaptive immune cells into the airway mucosa. We therefore speculate that CD23 is a critical receptor in the initiation of allergic responses in human airway inflammatory inflammation. Besides unraveling these basic mechanisms of CD23-mediated IgE and IC transport in humans, this study also suggests that blocking CD23-mediated IgE transport is a potentially important target to interfere with allergen-induced respiratory symptoms.

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Disclosures

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