The High Affinity IgE Receptor FcεRI Is Expressed by Human Intestinal Epithelial Cells

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Abstract

Background: IgE antibodies play a paramount role in the pathogenesis of various intestinal disorders. To gain insights in IgE-mediated pathophysiology of the gut, we investigated the expression of the high affinity IgE receptor FcεRI in human intestinal epithelium.

Methodology/Principal Findings: FcεRI α-chain, as detected by immunohistochemistry, was positive in epithelial cells for eight of eleven (8/11) specimens from colon cancer patients and 5/11 patients with inflammation of the enteric mucosa. The FcεRIα positive epithelial cells co-expressed FcεRIγ, whereas with one exception, none of the samples was positive for the β-chain in the epithelial layer. The functionality of FcεRI was confirmed in situ by human IgE binding. In experiments with human intestinal tumor cell lines, subconfluent Caco-2/TC7 and HCT-8 cells were found to express the α- and γ-chains of FcεRI and to bind IgE, whereas confluent cells were negative for γ-chains.

Conclusions/Significance: Our data provide the first evidence that the components of a functional FcεRI are in vitro expressed by the human intestinal epithelial cells depending on differentiation and, more importantly, in situ in epithelia of patients with colon cancer or gastrointestinal inflammations. Thus, a contribution of FcεRI either to immunosurveillance or pathophysiology of the intestinal epithelium is suggested.

Introduction

Although immunoglobulins are important constituents of host defense in mucosal compartments [1], they have been ascribed opposing functions in various intestinal diseases. Increased levels of immunoglobulin E (IgE) have been found during parasite infection with a putative beneficial host defense function [2,3]. In contrast, IgE plays a documented detrimental role in allergy. Significantly increased levels of IgE and anti-IgE autoantibodies might contribute also to the pathophysiology in Crohn’s disease (CD) [4]. Interestingly, it has been suggested that food allergic reactions might be triggered as a consequence of gastrointestinal inflammation [5,6]. Additionally, growing evidence points towards a participation of IgE in antibody-dependent tumoricidal activities [7–9].

IgE function depends on its interaction with effector cells via specific surface-receptors. The high affinity IgE receptor (FcεRI) is a multimeric cell-surface receptor, which binds the Fe domain of IgE with an affinity of 10^10 M^-1 [10]. The conformational change of the IgE constant region that occurs upon binding to FcεRI was proposed to contribute to the remarkably slow dissociation rate of receptor-bound IgE [11]. FcεRI has been so far detected on human mast cells, basophils, neutrophils, monocytes, macrophages, dendritic cells, Langerhans cells, eosinophils and platelets [12]. While the extracellular domain of the receptor α-chain carries the IgE binding site [13], the β- and γ-chains are involved in signal transduction [14,15]. The γc peptide tetramer is expressed in effector cells such as mast cells and basophils, and ligand-engagement leads to cell activation by a defined signaling cascade. In contrast, the αc trimer participates in antigen presentation [16].

The low affinity IgE receptor (FceRII/CD23) is a single chain glycoprotein with a molecular weight of 49 kDa [17]. In contrast to FcεRI, CD23 binds IgE with a significantly lower affinity (10^7 M^-1). CD23 was initially identified on B-lymphocytes [18] but subsequently also detected on various other cell types such as monocytes, macrophages, eosinophils and Langerhans cells [17,19,20]. Interestingly, CD23 is also expressed on intestinal epithelial cells where it is elevated in inflammatory conditions such as...
as CD and food allergies [21]. An IgE/CD23-dependent, transepithelial shuttle mechanism, regulated by interleukin (IL)-4, has been described, which mediates transport of intact food antigens [22–24].

Besides FcεRI and FcεRII/CD23, the IgE-binding protein (εBP, Galectin-3) also specifically interacts with IgE [25]. Due to its wide tissue distribution and expression on various cell types [26], a multifunctional role in cell growth regulation, cell adhesion and tumor metastases, among others, was suggested [27–29]. The intestinal distribution pattern of εBP is well established and it has been shown that it is downregulated in inflammation, whereas an elevated expression in colon cancer influences the neoplastic progression [30,31].

The presence of CD23 and εBP on intestinal epithelia is well documented, and functional studies have supported their biological importance. However, since no data were available concerning expression of FcεRI on enterocytes to date, we screened the intestinal mucosa of patients with gastrointestinal pathologies and controls, as well as intestinal epithelial cell lines for FcεRI expression. Herein, we report that both FcεRI α- and γ-chains are expressed by intestinal epithelial cells, while FcεRI β-chain could only be detected in the subepithelial stroma. The IgE binding found in α- and γ-chain positive tissues indicates the presence of a functional trimeric receptor FcεRIαγδ in human intestine, which could contribute to IgE-mediated pathophysiology of the gut.

Results

The High Affinity IgE Receptor FcεRI α- and γ-Chains Are Expressed on the Epithelium of Human Intestinal Tissue

Immunohistochemical (IHC) analysis revealed positive staining for FcεRI α-chain in the intestinal epithelium in eight of eleven (8/11) (73%) colon cancer patients and in 5/11 (45%) patients with gastrointestinal inflammation. In most cases, both small intestine and colon were positive for FcεRI α-chain. However, one patient per group (No. 24 and 12, respectively) was positive only in the colon, and two tumor patients (No. 21 and 26) expressed FcεRI α-chain only in the small intestinal epithelium. The four control samples from patients without gastrointestinal disorders were negative for FcεRI α-chain, suggesting that receptor expression might be observed only with gastrointestinal pathology (Table 1). Figure 1 shows IHC analysis of tissue sections from a representative colon cancer (A–F) and a gastrointestinal pathology (Table 1). Figure 1 shows IHC analysis from the tumor patient (No. 16), the FcεRI α-chain was only detected in the subepithelial stroma. The IgE binding found in α- and γ-chain positive tissues indicates the presence of a functional trimeric receptor FcεRIαγδ in human intestine, which could contribute to IgE-mediated pathophysiology of the gut.

Epithelial FcεRI Exhibits IgE Binding Properties

To determine whether the FcεRIαγδ expressed in the intestinal epithelium had specific antibody binding capacity, we performed IgE binding experiments on paraffin-embedded intestinal tissue sections. Samples positive for FcεRIαγδ on the apical cellular membrane (Figure 4A) showed specific binding of human serum IgE or humanized anti-4-hydroxy-3-nitrophenylacetyl (NP)-specific IgE (Figure 4B), with indistinguishable binding patterns. No epithelial binding was seen with unspecific human IgG antibodies or FITC-labeled dextran used as controls (data not shown). The observed IgE-FcεRI interactions correlated with the FcεRI α-chain expression in the epithelium, being expressed on the epithelial membrane as well as in the cytoplasm (Figure 4A, B). The IgE binding was not inhibited by anti-CD23 preincubation or by lactose, indicating protein- rather than carbohydrate-mediated IgE binding.

High Levels of FcεRI α- and γ-Chain mRNA Expression Are Detected in Intestinal Epithelial Cell Lines

To analyze the expression of the FcεRI complex on the transcriptional level in human intestinal epithelial cells, total RNA was isolated from subconfluent and confluent Caco-2/TC7 and HCT-8 cell lines. Human HMC-1 mast cells and transfected RBL-SX38 cells were used as positive controls, because they are known to abundantly express FcεRI α-, β- and γ-chains. Real-time PCR analysis revealed the presence of the FcεRI α-chain mRNA in all four intestinal cell lines, with highest expression in confluent HCT-8 cells (Figure 5A). The expression level was
approximately half that observed in the HMC-1 positive control cells (data not shown). The γ-chain mRNA also was found in all four intestinal cell samples, with highest levels detected in confluent Caco-2/TC7 cells (Figure 5B). In this case, the mRNA expression level of FcγRI expression was 80-times higher in the HMC-1 positive control cells (data not shown). None of these intestinal cell lines expressed detectable FcγRI β-chain mRNA (data not shown). In the second post-confluent cell line, high mRNA levels of all three FcγRI chains were observed, reflecting that these transfected RBL-SX38 cells over-express FcγRI (data not shown). Together, these findings verify the presence of FcγRI γ- and γ-chain mRNA, the components of the trimeric FcγRIγ2 complex, in human intestinal epithelial cells.

Highest Expression of FcγRI α- and γ-Chains Is Observed in Undifferentiated, Subconfluent Intestinal Cell Lines

To screen intestinal epithelial cell lines for FcγRI α-, β- and γ-chain protein expression we performed IF staining. We observed abundant α-chain expression in undifferentiated, subconfluent Caco-2/TC7 (Figure 6A, B) and HCT-8 cells (Figure 6E, F). To exclude intracellular staining signals for FcγRIα, we confirmed membrane integrity by comparing Triton-X-100 permeabilized cells with untreated controls using lamin A/C staining (Figure 6I, J). The total (Figure 6A) and surface-membrane expression (Figure 6B) were comparable in Caco-2/TC7 (Figure 6A, B) and HCT-8 cells (Figure 6E, F). To exclude intracellular staining signals for FcγRIα, we confirmed membrane integrity by comparing Triton-X-100 permeabilized cells with untreated controls using lamin A/C staining (Figure 6I, J). The total (Figure 6A) and surface-membrane expression (Figure 6B) were comparable in Caco-2/TC7 (Figure 6A, B) and HCT-8 cells (Figure 6E, F).

| Pat. No. | Sex | Age | Pathology (Tumor staging) | Small intestine | Colon | Lesion/tumor |
|----------|-----|-----|---------------------------|----------------|-------|-------------|
|          |     |     |                           | FcγRI-α Def. 5 | FcγRI-α Def. 5 | FcγRI-α Def. 5 |
| 1        | f   | 41  | – (obese patient)         | –              | +     | –           |
| 2        | f   | 39  | – (obese patient)         | –              | +     | –           |
| 3        | f   | 19  | – (biopsy, diarrhea, meteorism) | – | + | – |
| 4        | f   | 50  | –                          | –              | +     | –           |
| 5        | m   | 47  | Crohn’s disease, active phase | + Pc, M, sV + | + | + + + + + |
| 6        | f   | 35  | Crohn’s disease, active phase | –              | – | – + + + + + |
| 7        | f   | 27  | Crohn’s disease, active phase | –              | – | – + + + + + |
| 8        | f   | 37  | Crohn’s disease, active phase | + Pc, M, V + | + | + + + + + |
| 9        | m   | 19  | Crohn’s disease, active phase | –              | – | – + + + + + |
| 10       | f   | 61  | Crohn’s disease, active phase | –              | – | – + + + + + |
| 11       | m   | 28  | Crohn’s disease, active phase | –              | – | – + + + + + |
| 12       | f   | 29  | Crohn’s disease, active phase | –              | + | + M – + + + |
| 13       | m   | 50  | Diverticulitis and Peridiverticulitis | –              | – | – + + + + + |
| 14       | f   | 82  | Inflammation                | + Pc, M, V + | + | + + + + + |
| 15       | f   | 30  | C-Gastritis (biopsy)        | + Pc           | n.a. | n.a. n.a. n.a. n.a. |
| 16       | m   | 66  | Invasive, moderately well differentiated adenocarcinoma, ascending colon (G2, pT3, pN2, pM1, Dukes D) | + Pc, M, V + | + | + + + + + |
| 17       | f   | 78  | Invasive, moderately well differentiated adenocarcinoma, ascending colon (G2, pT2, pN1, Dukes C1) | –              | – | – + + + + + |
| 18       | f   | 69  | Invasive, moderately well differentiated adenocarcinoma, Cecum (G2/G3, pT3, pN1, Dukes C) | –              | – | – + + + + + |
| 19       | f   | 67  | Invasive, low differentiated adenocarcinoma, ascending colon (G3, pT3, pN0, pM1) | + Pc          | – | + + + + + |
| 20       | m   | 76  | Invasive, low differentiated adenocarcinoma, ascending colon (G3, pT3, pN0, pM0, V1) | + Pc, M, V + | + | + + + + + |
| 21       | m   | 84  | Invasive, well differentiated adenocarcinoma, ascending colon (G1, pT2, pN0, pM0, Dukes B) | + Pc, sV     | – | + + + + + |
| 22       | m   | 70  | Invasive, moderately well differentiated adenocarcinoma, ascending colon (G3, pT3, pN2, pM0, Dukes C1) | –              | + | – + + + + + |
| 23       | m   | 68  | Invasive, moderately well differentiated adenocarcinoma, Cecum (G2, pT3, pN0, pM0, Dukes B) | + Pc, M     | – | + + + + + |
| 24       | m   | 57  | Invasive, moderately well differentiated adenocarcinoma, Cecum (G2, pT4, pN0, V1, Dukes B) | –              | + | + + + + + |
| 25       | m   | 79  | Invasive, moderately well differentiated adenocarcinoma, ascending colon (pT3, pN0, pM0, Dukes B) | + Pc, M     | + | + + + + + |
| 26       | f   | 86  | Invasive, moderately well differentiated adenocarcinoma, ascending colon (pT4, pN2, pM1, V1, L1, Dukes D) | + Pc, V     | + | + + + + + |

n.d., not determined; n.a., not applicable; Pc, Paneth cell staining; M, Membrane staining; sV, single cell staining in villi; V, all villus epithelium.
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permeabilized cells (Figure 6E). The expression levels decreased with confluence, as only a faint staining could be detected in the cytoplasm of confluent Caco-2/TC7 (Figure 6C) and HCT-8 cells (Figure 6G). No FcεRIα positive staining was observed on the membrane of confluent Caco-2/TC7 cells (Figure 6D). In the

HCT-8 cells grown as multilayers with the new cells revealing a more immature phenotype, a scarce positivity was seen (Figure 6H).

We could not detect FcεRIβ in any intestinal epithelial cell line irrespective of the state of confluence (Figure 7A, D, G, J). In

Figure 1. Positive staining for FcεRI α-chain and defensin-5 is observed in serial sections from intestinal tissue. (A) FcεRI α-chain is detected on the membrane, as well as in the cytoplasm of epithelial cells in small intestine of cancer patient No. 16. FcεRI α-chain positive cells are also found in (B) the colon and (C) a tumor sample from the same patient. (D) Staining with anti-defensin-5 antibodies confirmed that FcεRIα expressing cells at the basis of the small intestinal crypts are Paneth cells. Defensin-5 is expressed also in (E) colon and (F) tumor sample in same areas, but to a lesser extend when compared with FcεRI α-chain staining. Similar staining pattern are observed also for the CD patient No. 8, as FcεRI α-chain positive cells are detected in (G) the epithelium of small intestinal tissue, (H) colon and (I) lesional region. Defensin-5 positive cells are located at (J) the crypt basis of small intestine, (K) along the colon crypt, as well as in (L) the lesional region. Original magnification ×10, inset ×40.

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Figure 2. FcεRI α-chain is expressed in epithelial cells. Co-staining with anti- FcεRI α-chain (red) and anti-keratin 8 antibodies (green) verifies the epithelial expression of FcεRI in (A) the crypts of small intestinal section, where FcεRIα is found primarily in the supranuclear region, in (B) colon tissue and in (C) tumor sample of cancer patient No. 16. (D) Negative control with mouse IgG2b and rabbit IgG. The blue fluorescence DAPI staining indicates the nuclei. Original magnification ×40.

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contrast, a prominent FcεRI γ-chain expression was seen in subconfluent Caco-2/TC7 (Figure 7B) and HCT-8 cells (Figure 7H), but not after confluence (Figure 7E, K). Consistent with these results, binding of IgE was high in the subconfluent Caco-2/TC7 (Figure 7C) and HCT-8 cells (Figure 7I), whereas only a background staining was seen in confluent Caco-2/TC7 (Figure 7F) and HCT-8 (Figure 7L) cells. The IgE binding was not inhibited by anti-CD23 preincubation.

The IF staining of Caco-2/TC7 and HCT-8 cells was confirmed by western blot analysis with cell lysates obtained from subconfluent and confluent cells. We observed a 45-kDa protein band of FcεRIα-chain in all cell lysates, with a stronger signal in

**Table 2.** FcεRI β- and γ-chain staining results in FcεRI α-chain expressing intestinal tissue.

| Pat. No. | Small intestine | Colon | Lesion/tumor |
|----------|-----------------|-------|--------------|
|          | FcεRI-α         | FcεRI-β | FcεRI-γ | FcεRI-α | FcεRI-β | FcεRI-γ | FcεRI-α | FcεRI-β | FcεRI-γ |
| 5        | +               | –      | +       |        |        |        |        |        |        |
| 8        | +               | –      | +       |        |        |        |        |        |        |
| 14       | +               | –      | +       |        |        |        |        |        |        |
| 15       | +               | –      | –       | n.a.   | n.a.   | n.a.   | n.a.   | n.a.   | n.a.   |
| 16       | +               | –      | +       |        |        |        |        |        |        |
| 19       | +               | –      | +       |        |        |        |        |        |        |
| 20       | +               | –      | +       |        |        |        |        |        |        |
| 21       | +               | –      | +       |        |        |        |        |        |        |
| 23       | +               | –      | +       |        |        |        |        |        |        |
| 25       | +               | –      | +       |        |        |        |        |        |        |
| 26       | +               | –      | +       |        |        |        |        |        |        |

n.a., not applicable;
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Figure 3. Co-expression of FcεRI α- and γ-chain, but not FcεRI α- and β-chain is observed in epithelial cells of intestinal tissue. In sections from cancer patient No. 23 FcεRI γ-chain (green) is found to be co-expressed in FcεRIα-positive epithelial cells of (A) the small intestine, as well as in (B) colon tissue and (C) tumor sample. In addition, sections from CD patient No. 8 are double-positive for FcεRI α- and γ-chain in (D) the small intestinal, (E) colon and in (F) lesional tissue. (G) Only in the subepithelial tissue FcεRI β-chain (green) positive cells are detected as shown here in the crypts of small intestinal tissue. (H) Negative control with mouse IgG2b and goat IgG isotype control antibodies. The blue fluorescence DAPI staining indicates the nuclei. Original magnification ×64.
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the subconfluent cells (Figure 8A). The band was rather sharp, even though FcεRI α-chain is known to be glycosylated. In contrast, we could detect the 10 kDa protein band of FcεRI γ-chain only in subconfluent Caco-2/TC7 and HCT-8 cells (Figure 8B). Both the α- and γ-chains were detected in the positive control RBL cell line over-expressing the human FcεRI (Figure 8B). High amounts of expressed FcεRI α- and γ-chain proteins were seen in the human mast cell line HMC-1 (data not shown). Thus, our data indicate that a functional high affinity IgE receptor is expressed by these cell lines when they are subconfluent and not after confluence.

Discussion

In the present study, we investigated the expression of FcεRI in the human intestinal epithelium and analyzed the role of cell growth or confluency on receptor expression in intestinal epithelial cell lines. We observed pronounced FcεRI α-chain expression in 73% of colon cancer patients and in 45% with gastrointestinal inflammations. In vitro experiments revealed only undifferentiated, subconfluent intestinal epithelial cells to express the α- and γ-chains of FcεRI. The functionality of the receptor was evident by the specific binding of IgE to the FcεRI α-chain.

It is easy to speculate that IgE antibodies might contribute to gastrointestinal inflammatory disorders [4–6]. The expression of the IgE high affinity receptor in cancer patients could have beneficial or detrimental effects. On the one hand, IgE antibodies have been suggested to participate in tumor immunosurveillance [9]. However, other facts could argue for a role of FcεRI in tumorigenesis. IgE binding to FcεRI was previously reported to both stabilize the receptors on the surface leading to receptor accumulation [32], but also to enhance survival of FcεRI bearing mast cells by autocrine cytokine secretion and induction of an anti-apoptotic protein [33]. Additionally, FcεRI signaling induces Ras activity, which in mast cells is associated with cell growth, differentiation and survival. Similarly, Ras initiates neoplastic cells proliferation via the inositol (1,4,5) triphosphate 3-kinase and ERK signaling [34,35]. Therefore, our data on FcεRI expression in colon tumor specimens, as well as in subconfluent, proliferating cell lines, might reflect a role for this receptor in tumor cell growth and survival.

It is well known that intestinal epithelial cells express HLA class II antigens on their surface and therefore are able to function as antigen presenting cells [36,37]. Interestingly, the cross-linking of trimeric FcεRIγ2 expressed on professional antigen presenting cells [12] by IgE and bound antigen was shown to induce endocytosis of the complex and uptake into MHC class II-rich compartments [16]. In this context, in IgE mediated disorders such as allergy a more enhanced efficiency of antigen-presentation to T cells was reported [38,39]. Thus, expression of FcεRI might contribute to the antigen presenting function of intestinal epithelial cells.

It is also possible that intestinal epithelial expression of FcεRI could exert a shuttle function for specific IgE through the intestinal epithelium. In diseases with elevated levels of IgE antibodies an increased intestinal transepithelial transport of IgE antibodies has been demonstrated, resulting in detectable amounts of IgE antibodies in feces [40]. From animal experiments, it is known that this IgE transport to the lumen occurs through intact

Figure 4. FcεRI positive tissue reveals IgE binding activity. Immunofluorescence staining of serial sections from patient No. 8 revealed IgE binding in (A) FcεRI α-chain (red) positive epithelial cells being incubated with (B) monoclonal NiP-specific humanized IgE antibodies (green). (C) Negative control with PBS. The blue fluorescence DAPI staining indicates the nuclei. Original magnification ×40.

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Figure 5. Expression of FcεRI α- and γ-chain but not β-chain mRNA in human intestinal epithelial cells. The expression pattern of the FcεRI complex was analyzed by real-time PCR analysis using specific primers for detection of (A) FcεRI α-, β- (not shown), and (B) γ-chain. Target gene expression levels were normalized to the average of housekeeping genes and are depicted relative to the value of subconfluent Caco-2/TC7 cells. The values are presented as means ± SD (n = 3) from one experiment. The results are representative of two independent experiments.

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epithelial barrier [41]. To date, only scant information is available regarding the mechanisms of this transepithelial IgE transport, even though antibody-binding structures seem to play a defined role. So far, only CD23 has been implicated in enhanced IL-4 dependent antibody shuttling through the intestinal epithelium [22,23]. The same IgE/CD23 shuttle mechanism seems to be responsible for transepithelial food allergen uptake and protect the antigens from degradation during this transport [24]. Based on the data presented here, FcεRI on enterocytes could contribute to IgE transfer through the gut epithelium. As no inhibition of IgE binding was seen in our experiments using anti-CD23 antibodies, the higher affinity of FcεRI to the Fc domain of IgE [10] could possibly enhance IgE transport.

Upon screening of the intestinal epithelium for FcεRI we found FcεRIα- but not γ-chain expression at the base of the crypts at the level of Paneth cells, as suggested by previous work of our group [42]. Interestingly, intracellular expression and cellular release of FcεRIγ was reported for other cell types such as eosinophils [43] and soluble IgE receptors were found to exert distinct biological functions such as regulation of IgE production, T cell and granulocyte maturation and macrophage migration [44]. Thus, Paneth cells might be of special interest as they contribute substantially to innate immunity in the intestine [45]. Further, Paneth cells appear to have a central role in some forms of inflammatory bowel disease [46]. Paneth cells are primarily present in small intestinal tissue in healthy individuals. However, metaplastic defensin-5 positive cells were found in the upper gastrointestinal tract and the diseased colon, and are considered an early marker of epithelial dysplasia and cancer development [47,48]. In contrast to the absorptive epithelial cells, which migrate from the crypts to the top of the villi during their 4–6 days lifespan [49], Paneth cells are found adjacent to the rapidly dividing pluripotent stem cells. Thus, alternative maturation signals are crucial for Paneth cell differentiation [50]. Even though real-time PCR analysis revealed the presence of the FcεRI α- and γ-chains mRNA in confluent intestinal cells, only undifferentiated proliferating cells expressed a functional FcεRIγγ as detected by cellular IF staining and Western blot experiments. This expression was decreased in confluent, differentiated cells. Thus, protein expression of FcεRI α- and γ-chain in intestinal epithelial cells might be mainly regulated on the translational level.

Our data demonstrate that the FcεRI α- and γ-chains, which are crucial components of the heterotrimeric FcεRIγγ isotype, are expressed in intestinal epithelial cells of patients with colon cancer or gastrointestinal inflammation. Although these data do not elucidate the precise function of FcεRI in the intestinal epithelium, the novel findings suggest that FcεRI may contribute to immunosurveillance or pathophysiology at the intestinal mucosa.

Materials and Methods

Patient Samples

Four-micron sections of formalin fixed (buffered formalin, pH 7.5) paraffin-embedded tissue of small intestinal and colon specimens were investigated. The samples were obtained from eleven patients with gastrointestinal inflammations and eleven patients with colon cancer. In each case, mucosa with pathological changes as well as regular mucosa without any histological signs of abnormality was investigated. Additionally, sections of biopsies

Figure 6. Abundant surface and cytoplasmatic FcεRI α-chain expression only in subconfluent human intestinal tumor cell lines. Immunofluorescence staining for (A–H) FcεRIα is performed in (A, B) subconfluent and (C, D) confluent Caco2/TC7 as well as in (E, F) subconfluent and (G, H) confluent HCT-8. Triton-X-100 permeabilized (A, C, E, G) and untreated cells (B, D, F, H) were compared. (I–L) Representative control staining in subconfluent Caco2/TC7 with the (I, J) anti-lamin A/C or (K, L) unspecific murine IgG2b, (I, K) permeabilized or (J, L) untreated. The blue fluorescence DAPI staining indicates the nuclei. Original magnification ×40.
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from four patients without gastrointestinal diseases served for control purposes. Patient characteristics are summarized in Table 1. The inclusion of human specimens for the present study was approved by the ethic’s committee of the Medical University Vienna (number: 317/2007). The protocol permitted only usage of numerically coded human tissue sections from therapeutic interventions. The authors of the study had no access to patients’ data except the information given in Table 1. Thus, the ethic’s committee of the Medical University Vienna waived informed consent.

Antibodies

We used the following anti-human primary antibodies in this study: mouse monoclonal anti-FcεRI α-chain (clone CRA1) purchased from Cosmo Bio (Co., Tokyo, Japan), rabbit polyclonal anti-FcεRI α-chain purchased from Upstate (Lake Placid, NY), mouse monoclonal anti-defensin-5 (clone 1G11) generated by C. L. Bevins [47], goat polyclonal anti-FcεRI β-chain, goat polyclonal anti-FcεRI γ-chain, mouse monoclonal anti-CD23 (clone BU38) and goat polyclonal anti-lamin A/C purchased from Santa Cruz Biotechnology Inc. (Santa Cruz CA), rabbit polyclonal anti-FcεRI γ-chain purchased from Abcam (Cambridge, UK), rabbit anti-keratin 8 purchased from NeoMarkers Inc (Fremont, CA), mouse monoclonal anti-actin purchased from Chemicon International, (Millipore, Billerica, MA); human serum IgE purchased from Alpha Diagnostic Intl. Inc. (San Antonio TX), humanized murine anti-NiP IgE antibody purchased from Serotec (Oxford, UK). Non-specific mouse IgG1 and IgG2b (Jackson ImmunoResearch Laboratories, West Grove, PA), non-specific goat IgG (Zymed Laboratories San Francisco, CA) and non-specific rabbit IgG (Serotec, Oxford, England) served as negative controls. Specific binding of antibodies was detected using the following secondary antibodies or detection reagents: goat anti-mouse AlexaFluor 568-conjugated and goat anti-rabbit AlexaFluor 488-conjugated purchased from Molecular Probes (Invitrogen, Carlsbad, CA), biotinylated rabbit anti-goat and FITC-Streptavidin purchased from Dako (Carpinteria, CA), FITC-labeled donkey anti-goat (Jackson ImmunoResearch Laboratories), FITC-labeled goat anti-human IgE purchased from Vector Laboratories (Burlingame, CA); HRP-conjugated anti-rabbit purchased from Amersham Biosciences (GE Healthcare, UK), HRP-conjugated anti-mouse purchased from Jackson Immunoresearch Laboratories. 4-hydroxy-3-nitrophenylacetyl.

Figure 7. Abundant FcεRI γ-chain expression and IgE binding in subconfluent human intestinal tumor cell lines. Immunofluorescence staining of (A, D, G, J) FcεRI β- and (B, E, H, K) FcεRI γ-chain are performed in (A–C) subconfluent and (D–F) confluent Caco2/TC7 and in (G–I) subconfluent and (J–L) confluent HCT-8. (C, F, I, L) According to the expression pattern of FcεRI in the subconfluent cells, IgE binding is observed exclusively in subconfluent, non-mature intestinal cells. The blue fluorescence DAPI staining indicates the nuclei. Original magnification x40. doi:10.1371/journal.pone.0009023.g007
staining with hematoxylin the sections were dehydrated and mounted with Eukitt, and then analyzed using a Nikon Eclipse E400 microscope (Nikon, Vienna, Austria).

**Immunofluorescent Double-Staining**

Sections were treated as described above with slight modifications. After antigen unmasking with 10 mM citrate buffer pH 6 and permeabilization with PBS/0.2% Tween, the sections were blocked with 5% goat serum. For the FcεRI α-chain/β-chain double staining, the first primary antibody applied was mouse anti-FcεRI α-chain (Cosmo Bio) overnight at 4°C. Mouse IgG2b served as negative control. After washing goat anti-mouse AlexaFluor 488 was applied for 1 h. After washing and blocking again with 5% goat serum, the second staining was performed using rabbit anti-keratin 8 as the second primary antibody (or rabbit IgG as negative control) for 1 h. After washing, goat anti-rabbit AlexaFluor 568 secondary antibody was applied for 1 h. For the double-staining of FcεRI β- or γ-chain the first primary antibody applied was goat anti-FcεRI β- or γ-chain overnight at 4°C after blocking with 5% rabbit serum. Goat IgG was used as negative control. After washing the sections were incubated with biotinylated polyclonal rabbit anti-goat Ig for 30 min at 37°C. The sections were again washed and incubated with FITC-Streptavidin for 30 min at room temperature. After washing and blocking again with 5% goat serum, the second staining was performed using the mouse anti-FcεRI α-chain as the second primary antibody (or mouse IgG2b as negative control) for 1 h. After washing goat anti-mouse AlexaFluor 568 was applied for 1 h. The sections were washed and counterstained with 4,6-Diamidino-2-phenylindole 0.1 μg/ml (Molecular Probes). Afterwards, sections were mounted in Vectashield medium (Vector Laboratories) and investigated in a Zeiss AxioPlan 2 microscope (Carl Zeiss Göttingen, Germany).

**Human IgE Binding in Intestinal Tissue Sections**

For IgE binding experiments, human intestinal sections were cleared in xylene, rehydrated and subjected to heat-mediated antigen retrieval as described above. Residual antibodies were stripped by low pH treatment, and unspecific binding sites were blocked with 5% BSA/PBS for 30 min. To block IgE binding to Galectin and CD23, α-Lactose 25 mM and anti-CD23 (clone BU38) were applied overnight at 4°C. Thereafter, slides were incubated with 10 μg/ml NiP-IgE, serum IgE or PBS for 2 h at 37°C. For controls, either 5 μg/ml FITC-labeled IgG or dextran in PBS/1% BSA were incubated overnight at 4°C under light protection. The sections were then incubated with either FITC-labeled NiP-IgE (NiP-IgE) or goat anti-human IgE (NiP-IgG and serum IgE). The sections were further processed for IF or IHC analysis as described above. Only the results generated with NiP-IgE/FITC-labeled anti-human IgE are shown.

**Cell Culture**

All cell culture media were supplemented with 10% heat inactivated FCS (PAA Laboratories, Pasching, Austria), 4 mM glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin if not explicitly stated. The human adenocarcinoma cell line HCT-8 (originating from the ileocecal region) (kindly provided by Gerhard Hamilton, Department of Surgery, Medical University Vienna, Vienna, Austria) was grown in RPMI 1640 supplemented with 10% FCS (PAA Laboratories, Pasching, Austria) and 4 mM glutamine. The human colon adenocarcinoma cell line Caco-2 clone TC7 (Caco-2/TC7) (kindly provided by Monique Roussel, INSERM, Paris, France) was grown in Dulbecco modified...
mineral essential medium + GlutaMAX-II with high glucose (4500 mg/ml), supplemented with 1% non-essential aminoacid, 10 mM HEPES. Cells were used at subconfluent stage (60% confluence) or 10 days after confluence. For all experiments RBL-SX38 cells which are transfected with the complete human FceRI (kindly provided by Jean-Pierre Kinet, Harvard Institute of Medicine, Boston, MA) and human HMC-1 mast cells (kindly provided by Joseph H. Butterfield, Mayo Clinic, Rochester, MN) served as a positive control. RBL-SX38 cells were cultured in RPMI 1640 medium, HMC-1 cells were grown in IMDM.

RNA Isolation, Reverse Transcription and Real-Time PCR Analysis

Total RNA was isolated from subconfluent and confluent human Caco-2/TC7 and HCT-8 intestinal cell lines by using the RNeasy RNA isolation Kit (Qiagen, Vienna, Austria), including on-column DNase digest following the manufacturer’s instruction. RNA integrity was analyzed by agarose gel electrophoresis. RNA concentration and purity was determined using a Biophotometer (Eppendorf, Hamburg, Germany). RNA isolated from HMC-1 and RBL-SX38 cells was used as a positive control for evaluation of target gene (human FceRI α-, β-, and γ-chain) expression. Two μg of total RNA were transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Vienna, Austria).

Expression analysis was performed by real-time PCR on a StepOne Plus real-time PCR system (Applied Biosystems). The analysis was carried out with a two step protocol starting with 20 seconds at 95°C, followed 40 cycles of 1 second at 95°C and subsequent 20 seconds at 60°C. In all experiments triplicates were set up containing the POWER SYBR Green PCR Master Mix (Applied Biosystems) reagent. Primers were designed using “Primer Express 2.x” software (Applied Biosystems) and span, when possible, exon-intron boundaries to avoid signals from contaminating genomic DNA. The forward (fw) and reverse (rv) primers consist of the following sequences: beta Actin fw: tgtgcctcggagacac, rev: ctttagtggctgacagtgta; RPLP0 fw: cctggaacctctactttgct, rev: ctttagtggctgacagtgta; FcêRIα fw: caggaactccatctcattcag, rev: gagaatgaaactattgctggag; FcêRIβ fw: caagcaggggagagc, rev: gagaatgaaactattgctggag; FcêRIγ fw: caagcaggggagagc, rev: gagaatgaaactattgctggag. Based on melting curve analysis no primer–dimer signals were generated during the PCR amplification. For relative quantification, data were analyzed by ΔΔCT method using “StepOne software 2.0” (Applied Biosystems). Expression levels of target genes were normalized to the average of house keeping genes, beta-actin and RPLP0, and are shown relative to the value of subconfluent Caco-2/TC7.

Cell Immunostaining

Cells grown on glass cover slips were fixed for 20 min in 3% paraformaldehyde. After washing, the cells were incubated for 15 min with 50 mM NH4Cl to reduce spontaneous fluorescence. Unspecific binding sites were subsequently blocked by incubating cells with 5% goat serum. Cells were treated with mouse monoclonal anti-FcêRI α-chain (clone CRA1), goat polyclonal anti-FcêRI β-chain or goat polyclonal anti-FcêRI γ-chain (Santa Cruz Biotechnology Inc.) overnight at 4°C. Unspecific Mouse IgG2b or goat IgG were used as negative control. After washing the cells incubated with anti-FcêRI α-chain were treated with goat anti-mouse AlexaFlour 568 for 1 h. The cells incubated with anti-FcêRI β-chain or anti-FcêRI γ-chain were treated with biotinylated polyclonal rabbit anti-goat Ig for 30 min at 37°C followed by FITC-Streptavidin for 30 min at room temperature. For the cellular IgE binding assay a blocking step with anti-CD23 antibodies (clone BU38) applied overnight at 4°C was followed by incubation with 10 μg/ml NiP-IgE, serum IgE or PBS for 2 h at 37°C. The cells were then incubated with either FITC labeled NiP-BSA (NiP-IgE) or anti-human IgE antibodies (NiP-IgE and serum IgE). The cells were further processed for immunofluorescence analysis as described above. Only the experiments conducted with NiP-IgE/FITC labeled anti-human IgE are shown.

To analyze for membranous or total expression of FcêRI α-chain, cells were either permeabilized with 0.2% Triton-X-100 for 30 min, or left with PBS. Goat anti-lamin A/C antibody was used as positive control.

Cell lysate and Western Blotting Analysis

Total protein cell extract and western blot analysis was performed as described before [52]. Briefly, after washing cells were homogenized in 1 ml lysis buffer (10 mM TRIS pH 7.4 containing 1% SDS). The lysate was boiled for 5 min, centrifuged at 10000 g, and then stored at −80°C. Equal amounts of protein were separated by 12% SDS-PAGE and blotted to a nitrocellulose membrane. Non-specific binding sites were blocked with dried milk powder in PBS/0.1% Tween. The antibodies used were rabbit polyclonal anti-FcêRI α-chain (Upstate), rabbit polyclonal anti-FcêRI γ-subunit (Abcam), or mouse monoclonal anti-actin. As secondary antibodies HRP-conjugated anti-rabbit antibody (Amersham Biosciences) or HRP-conjugated anti-mouse antibody (Jackson ImmunoResearch Laboratories) were used. Bands were detected with the SuperSignal CL-HRP Substrate system (Pierce, Rockford, IL) and quantified by densitometry (EasyWin, Herolab, Wiesloch, Germany).

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Author Contributions

Conceived and designed the experiments: EU GB PS DEJJ. Performed the experiments: EU GB PS. Analyzed the data: EU GB PS. Contributed reagents/materials/analysis tools: CLB FW. Wrote the paper: EU GB PS CLB DEJJ.
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