Award Review

Interaction between the Intestinal Immune System and Commensal Bacteria and Its Effect on the Regulation of Allergic Reactions

Kyoko TAKAHASHI

Food and Physiological Functions Laboratory, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-8510, Japan

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The immune system and the commensal bacteria in the intestine, which together form the intestinal symbiotic system, greatly contribute to regulation of allergy. Of the various types of cells constituting the intestinal immune system, this review focuses on epithelial cells and mast cells and the interaction of these cells with commensals. Mast cells express the high affinity IgE receptor FcεRI which is essential to the induction of allergic inflammatory reactions. The molecular mechanisms of transcriptional regulation of genes encoding FcεRI have been clarified. On the other hand, the expression of the molecules involved in microbe recognition is regulated in a specific manner in intestinal epithelial cells, which are continuously exposed to the commensals inhabiting the intestinal lumen, to prevent excessive inflammatory reactions. Microbial components directly regulate the functions of mast cells through Toll-like receptors. These aspects provide targets for the regulation of allergy based on the maintenance of the intestinal symbiotic system.

Key words: allergy; intestinal immune system; intestinal commensal bacteria; mast cell; intestinal epithelial cell

It is a serious social problem that a large and increasing population suffers from some form of allergy. Since allergy is an immune disorder with a complicated pathophysiology, multilateral approaches are required for prevention and cure. The role of the intestinal immune system, the largest immune system in the body, and of the commensal bacteria inhabiting the intestinal tract in the regulation of allergy has lately attracted considerable attention. The intestinal immune system accurately recognizes commensal bacteria, pathogenic bacteria invading the intestine, and food antigens, discriminates between safe or beneficial and dangerous or hazardous components, and attacks only those that are hazardous to the host (Fig. 1). Although the commensal bacteria are not immunologically "self" to the host, the intestinal immune system does not exclude them completely. Rather, the immune system and the commensal bacteria form the symbiotic system in the intestine.

Maintenance of the symbiosis between the intestinal immune system and the commensals is required for intestinal homeostasis. On the other hand, disorder in this intestinal symbiotic system is thought to increase disease rates and to aggravate the symptoms of allergy.

In this review, the following three topics concerning the relationship between the intestinal symbiotic system and allergy are discussed: (i) The expression of high affinity IgE receptor (FcεRI) in mast cells, (ii) The expression of Toll-like receptors (TLRs) and related molecules in intestinal epithelial cells (IECs), and (iii) The effects of bacterial components on the allergic reactions of mast cells. The first two have to do with the molecular mechanisms of transcriptional regulation of the genes related to allergic reaction (i) and microbe recognition (ii) in the intestine as a fundamental mechanism supporting symbiosis. The third regards the involvement of commensal bacteria in the regulation of allergic reactions in the intestine.

I. Regulation of FcεRI Expression in Mast Cells

The high affinity IgE receptor plays a key role in inducing allergic reactions. Cross-linking of FcεRI on mast cells by antigen (allergen)-IgE complexes activates intracellular signal cascades and triggers degranulation, leukotriene and prostaglandin secretion, and cytokine production. FcεRI is composed of three different subunits, α, β, and γ, the α-chain of which directly binds IgE through its extracellular domain, while the β and γ-chains are responsible for mediating intracellular signals.

Although a functional receptor is expressed both as tetramers (αβγ2) and trimers (αγ2) in humans,1,2 in addition to cell-surface expression of the receptor,3,4 have been reported to be significantly amplified by the β-chain, indicating that the β-chain increases cell activation sensitivity to stimulation by allergens. Recently, it was found that the β-chain amplified degranulation and leukotriene secretion but suppressed cytokine production.5,6 These findings indicate that the β-chain, a fine regulator of FcεRI-mediated...
cell activation, can serve as a target in the regulation of allergy. Since the /C12 cell activation, can serve as a target in the regulation of

Regulatory Mechanism of Fc

Fig. 1. The Intestinal Immune System.

The intestinal immune system tolerates commensal bacteria and food antigens as safe and beneficial components but excludes pathogenic bacteria as dangerous, hazardous components. Such precise discrimination by the intestinal immune system supports intestinal homeostasis.

Fig. 2. Regulatory Mechanism of FcRI β-Chain Gene Transcription.

HDACs are recruited to the β-chain gene through an element in the fourth intron by MZF-1/FHL3/NFY, repressing β-chain gene transcription by deacetylation of histones. GM-CSF downregulates β-chain gene expression in that it promotes the formation of the regulatory complex binding to the fourth intronic element by inducing upregulation of MZF-1 and nuclear translocation of FHL3. Modified from ref. 11.

also in the repression of β-chain gene expression in peripheral cells under specific circumstances.

On the other hand, the FcRI γ-chain, which is known to be essential to intracellular signal transduction, is also a constituent of other immunoglobulin Fc receptors, including the IgG receptors (FcγRI and FcγRIII)[2–5] and the IgA receptor (FcεRI).[6–9] The collagen receptor glycoprotein (GP) VI on platelets,[10] and the osteoclast-associated receptor (OSCAR) on dendritic cells and monocytes.[11–13] Therefore, FcεRI can serve as a target in the prevention of various immune diseases, such as thrombosis and lupus nephritis, in addition to allergy, in which unusual and excess activation of effector cells via the γ-chain is observed.

The human FcεRI γ-chain consists of five exons, and its 5’ region contains a GC box and a reversed CAAT box, but not a canonical TATA box.[14] As seen in many genes possessing a TATA-less promoter, transcription of the FcεRI γ-chain starts at various positions including a major site 25 bp upstream of the translation start site and several minor sites within 100 bp of the translation start site. Brini et al. analyzed the 5’ region of the human FcεRI γ-chain over 2.5 kb and found that this region is involved in hematopoietic cell-specific transcriptional activation. We analyzed a 5’ region of about 450 bp of the human FcεRI γ-chain, and identified three cis-elements within 100 bp upstream of the translation start site. One of the cis-elements was recognized by transcription factor Sp-1, and another was recognized by GABP and by Elf-1. The sequence of the other element was similar to a binding motif of the C/EBP family. These transcription factors cooperatively regulate the FcεRI γ-promoter (Fig. 3). Sp1 and GABP synergistically activate the FcεRI γ-promoter. This synergistic activation perhaps requires physical interaction between the two transcription factors, because the Ets domain of GABP was found to bind Sp1 directly. On the other hand, GABP and Elf-1, whose recognition sequences overlap, were shown to bind the FcεRI γ-chain with similar affinity in the context of chromatin, although Elf-1 had weaker enhancer activity for FcεRI γ-chain expression than did GABP. Both transcription factors are thought to compete for binding to the cis-element, because additional expression of Elf-1 in combination with Sp1 and GABP reduced FcεRI γ-promoter activity. Such functional and
Various immune diseases involving FcR expression are promising targets for application against several human IEC lines. The expression levels of mechanisms of TLR4 gene expression in IECs using transcription start site. We analyzed the regulatory protein (ICSBP) regulate human TLR4 gene expression downregulation of TLR4 gene expression by these TLR4 gene transcription in IECs. It is thought that increased TLR4 mRNA expression, indicating that both histone deacetylase and DNA methyltransferase a LPS-high responder IEC line. Moreover, inhibition of region of the TLR4 gene was significantly higher in bacteria. Rehli which constitutes the cell wall of gram-negative bac-
to be regulated by cell type-specific mechanisms in IECs. Therefore, the expression of these molecules is thought to be regulated by cell type-specific mechanisms in IECs to maintain intestinal symbiosis.

TLR4 mainly recognizes lipopolysaccharide (LPS), which constitutes the cell wall of gram-negative bacteria. Rehli et al. have reported that transcription factors of PU.1 and interferon consensus sequence-binding protein (ICSBP) regulate human TLR4 gene expression in myeloid cells through elements just upstream of the transcription start site. We analyzed the regulatory mechanisms of TLR4 gene expression in IECs using several human IEC lines. The expression levels of TLR4 mRNA varied among the cell lines. Most of the IEC lines used in the experiment expressed smaller amounts of TLR4 mRNA than a monocyte line used as control, with the exception of one IEC line that expressed a high level of TLR4 mRNA. TLR4 mRNA expression in each IEC line was almost exactly correlated with its LPS responsiveness. Moreover, the transcriptional enhancing activity of the 5′ region of the TLR4 gene as measured by reporter gene assay in each IEC line was also correlated with the amounts of IL-8 secreted into the culture supernatant upon stimulation with LPS, indicating that the low responsiveness of human IEC lines to LPS was brought about mainly by downregulation of TLR4 gene transcription. Histone deacetylation, as well as DNA methylation, at the 5′ region of the TLR4 gene was significantly higher in LPS-low responder IEC lines than in a monocyte line or a LPS-high responder IEC line. Moreover, inhibition of both histone deacetylase and DNA methyltransferase increased TLR4 mRNA expression, indicating that epigenetic mechanisms in IECs prevents excessive inflammatory reaction at the intestinal mucosa.

The expression of microbe recognition molecules is also regulated at the post-translational level, in addition to the transcriptional level as seen for the TLR4 gene, controlling responses to the microbe of IECs. For example, TLR5, which recognizes flagellin, the structural component of bacterial flagella, is highly expressed only on the basolateral side of IECs. It is thought that pathogenic bacteria possessing flagella pass through the cytosol of IECs to the basolateral side, are recognized there by TLR5, and induce inflammatory reactions. Some commensal bacteria also possess flagella, but they cannot pass through IECs to reach the basolateral side. On the other hand, TLR9, which recognizes bacterial CpG DNA, is detected on both the apical and the basolateral surface of IECs in addition to the intra-
cellular endosomal membrane. Stimulation of surface TLR9 on the apical side, but not on the basolateral side, activates IECs, inducing inflammatory reactions. Moreover, stimulation of apical TLR9 inhibits the activation of basolateral TLR9, suggesting an inhibitory role of apical TLR9 in intestinal inflammation. In this case, the intracellular distribution of signaling molecules might be polarized. These regulations of microbe recognition molecule expression in IECs by control of gene transcription and intracellular localization possibly contribute to the control of allergy through the main-
tenance of intestinal homeostasis.

### III. Effects of Bacterial Components on the Allergic Reactions of Mast Cells

Lately, much attention has been focused on the effects of intestinal commensal bacteria on the regulation of allergy. A correlation between the incidence of atopic eczema in children and the composition of their intestinal microbiota has been reported. In addition, the potential of probiotics such as _Lactobacillus_ and _Bifidobacterium_, defined as live microorganisms that confer health benefits on the host, to prevent or alleviate the symptoms of allergy is of interest. It has been proposed with regard to several large-scale randomized double-blind controlled trials that probiotic mi-
crobes prevent atopic eczema in infants. Unequivocal evidence, however, has not been provided to demon-
strate suppression of allergy by probiotics, probably because of the complicated pathophysiology of allergy, differences in the bacterial strains employed in various experiments, and the mildness of the effects.

The effects of orally administered probiotics are thought to be exerted through influences on both intestinal microbiota and the intestinal immune system. In spite of numerous reports on the anti-allergic effects of probiotics, their mode of action is not yet fully understood. In animal studies, a decrease in serum IgE levels supported by an improvement in the Th1/Th2 balance or induction of regulatory T cells is generally believed to be associated with the suppression of allergy by probiotics. In ovalbumine (OVA)-specific T cell receptor transgenic mice, intestinal bacteria were shown to be involved in the regulation of OVA-specific responses induced by oral administration of OVA, such as a decrease in cytokine production and induction of
regulatory T cells. On the other hand, clinical symptoms are often alleviated without a decrease in serum IgE levels in human trials, suggesting that different mechanisms, independent of IgE sensitization, also work. Mast cells, which express FcεRI on their surfaces and play an essential role in inducing allergic inflammation in peripheral tissues, are potential targets of probiotics given the recent discovery that they express TLRs.

We examined to determine whether microbial components directly influence the functions of mast cells through TLR2, which is mainly known to recognize cell wall components of gram-positive bacteria, because most probiotic bacteria, such as *Bifidobacterium* and *Lactobacillus*, belong to the Gram-positive group. Pretreatment of IgE-sensitized mast cells with *Bifidobacterium pseudocatenulatum* JCM 7041, isolated from human feces, suppressed degranulation upon antigen stimulation in vitro. The synthetic TLR2 ligand Pam3CSK4 also suppressed leukotriene C4 production, as well as degranulation, triggered by the engagement of FceRI. Intracellular Ca2+ mobilization and phosphorylation of Erk were suppressed by pretreatment with Pam3CSK4, suggesting that the TLR2 ligand suppresses activation of mast cells by interrupting FceRI-mediated intracellular signaling. Furthermore, the effects of Pam3CSK4 on mast cell-induced increases in vascular permeability in vivo were investigated by employing mast cell-deficient W/Wv mice into which IgE-sensitized mouse bone marrow-derived mast cells (BMMCs) were transferred. Pam3CSK4 treatment of BMMCs reduced the increase in vascular permeability in the recipient W/Wv mice upon intravenous injection of antigen in a TLR-dependent manner. These results suggest that probiotics exert potential anti-allergic effects, at least in part through direct effects on mast cells. Recently, in addition to their role as effector cells in allergy, the protective role of mast cells during acute infection has been featured as their primary physiological function. Analyses of the influence of microbial components on mast cells will help us not only to optimize probiotic use for anti-allergy purposes but also to elucidate the contribution of commensal bacteria to mast cell functions in the intestine.

IV. Concluding Remarks

Control of the interaction between the intestinal immune system and intestinal commensal bacteria is a key factor in regulating allergy. The genes involved in maintenance of the homeostasis of the intestinal symbiotic system, including those engaged in microbial recognition and those directly engaged in induction of allergic reactions in epithelial cells and mast cells are potential targets for regulating allergic inflammation (Fig. 4).

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References
