

MAST CELLS: LINKING ALLERGY AND MICROBIOME

PAUL FORSYTHE

Firestone Institute for Respiratory Health and Department of Medicine,
McMaster University, Hamilton, Ontario, Canada.

SUMMARY

It is now widely accepted that there is a relationship between the microbiota and development and severity of allergic disease. In investigating the mechanisms underlying microbiota modulation of allergic disease the focus has been on the induction phase of the disease; alterations in the phenotype and function of antigen presenting cells, induction of regulatory T cells and shifts in Th1/Th2 balance. However there is evidence that microbes can influence the effector phase of allergic disease. The position of mast cells on the frontline of defence against pathogens also suggests they may play an important role in fostering the host-microbiome relationship and maintaining the dynamic ecosystem of the super-organism. There is emerging evidence that the mast cell plays an important role in microbiota host communication and in particular, that certain non-pathogenic microbes and components of the microbiota can influence the development and severity of allergic disease by modulating mast cell function. Furthermore, it appears that different non-pathogenic bacteria can utilize distinct mechanisms to stabilize mast cells, acting locally though direct interaction with the mast cell at mucosal sites or attenuating systemic mast cell dependent responses, likely thorough indirect signalling mechanisms. Further investigation of mast cell regulation by non-pathogenic or commensal bacteria will likely lead to a greater understanding of host microbiota interaction and the role of the microbiome in development of allergy and other diseases.

INTRODUCTION

The Mast cell, long regarded as a key effector of human allergic disease and immune responses to helminthic parasites is now considered a primary inducer and amplifier of both innate and adaptive immune responses (*Vliagoftis and Befus, 2005*).

The presence of mast cells in particularly high numbers in the skin, airway and gut mucosa is indicative of a role as important gatekeepers/sentinels for fighting infectious organisms in these portals of entry. MC express a complement of Toll-like and other microbe

associated pattern recognition receptors and activation by bacteria leads to signals that modulate both innate and adaptive immune responses that protect against infection. Mast cells participate in direct killing of organisms by phagocytosis and reactive oxygen species production and can produce anti-microbial peptides such as cathelicidins (*St John and Abraham, 2013*). Furthermore, mast cells have been found to produce extracellular traps that encompass and kill organisms in a similar manner to neutrophils (*von Kockritz-*

Blickwede et al., 2008). Mast cells also protect against infection indirectly processing and presenting bacterial antigens to T cells and recruit other inflammatory cells through the release of pro-inflammatory mediators (*Moon et al.*, 2010).

Increased study and awareness of the important physiological role of the microbiota is leading to a shift in our perceptions of immunology. A realization that the relationship between the immune system and the external environment is not purely adversarial and that the immune system has evolved not only to protect against pathogens

but, just as importantly, to promote a healthy microbiota. Thus the position of mast cells on the frontline of defence against pathogens also suggests they may play an important role in fostering the host-microbiome relationship and maintaining the dynamic ecosystem of the super-organism. There is emerging evidence that the mast cell plays an important role in microbiome host communication and in particular, that certain non-pathogenic microbes and components of the microbiota can influence the development and severity of allergic disease by modulating mast cell function.

MICROBIOTA AND ALLERGY: A ROLE FOR MAST CELLS?

The ‘microflora hypothesis’ proposes that perturbations in microbiota, because of dietary changes and increased antibiotic use in ‘industrialized’ countries, leads to disruption of the normal microbiota-mediated mechanisms of immunological tolerance in the mucosa. Consequently this dysregulation leads to an increased susceptibility to immunological disorders including allergy (*Noverr and Huffnagle*, 2005; *Shreiner et al.*, 2008). Recent studies utilizing gnotobiotic mice have identified that key species within the microbiota, such as clostridia (*Atarashi et al.*, 2011), single bacterial strains, or isolated components of these strains, can strongly influence the development of T cell phenotypes in the intestine (*Round and Mazmanian*, 2010). Overall evidence suggests that the microbiota of various tissue sites play a central role in governing susceptibility to the Th2 skewed inflammatory response associated with allergy. Certain components of microbiota are immunomodulatory, and colonization of specific subsets of microbes can induce immune signals that are ei-

ther pro-inflammatory or tolerogenic in nature. Studies in animal models also lend support to the microflora hypothesis. In particular, a recent study by *Novall Rivas et al.* (2013) identified that mice genetically predisposed to food allergy (I14raF709) exhibit a specific gut microbiota signature distinct from sensitization-resistant wild-type mice. Strikingly, adoptive transfer of microbiota from I14raF709 but not wild type mice into naïve recipients lead to up-regulation of OVA-specific TH₂ and IgE responses and promoted anaphylaxis. This strongly suggests that dysbiosis provides one mechanism contributing to the pathogenesis of allergy.

Strong evidence for a causal relationship between specific microbiota changes and allergy comes from the study of the skin and allergic dermatitis. Overall, the skin of atopic dermatitis patients has been demonstrated to have greatly reduced microbiota diversity relative to the same skin area of healthy controls. *Staphylococcus aureus*, rarely present on healthy skin is found on the skin in more than 90% of atopic dermatitis patients and some

treatments to reduce *S. aureus* colonization decrease disease severity (Leung et al., 2008). Furthermore a recent analysis of the microbiota composition of lesional skin of moderate-to-severe paediatric atopic dermatitis patients revealed temporal shifts associated with disease flares and treatment (Kong et al., 2012). Specifically, the relative abundance of *S. aureus* and the skin commensal *Staphylococcus epidermidis* was increased with disease severity, whilst *Streptococcus*, *Propionibacterium*, and *Corynebacterium* genera were increased following therapy (Kong et al., 2012).

Significantly, another recent study by Nakamura et al. (2013) identifies mast cells as a mechanistic link between *S. aureus* and atopic dermatitis. These investigators identified that δ -toxin, produced by *S. aureus*, was a

mast cell degranulating factor and that *S. aureus* isolates recovered from patients with atopic dermatitis produced large amounts of δ -toxin. Furthermore, immunoglobulin-E enhanced δ -toxin-induced mast cell degranulation in the absence of antigen. Skin colonization with *S. aureus*, but not a mutant deficient in δ -toxin, increased IgE and interleukin-4 production and promoted inflammatory skin disease. Crucially, the enhancement of IgE production and dermatitis by δ -toxin was abrogated in KitW-sh/W-sh mast-cell-deficient mice and restored by mast cell reconstitution (Nakamura et al., 2013). Thus it appears that *S. aureus* infection drives a Th2 type immune response with associated IgE and inflammatory cytokine production through direct action on mast cells.

COMMENSAL BACTERIA REGULATE MAST CELL FUNCTION

It has emerged in recent years that microbial exposure either naturally in the environment or through dietary supplementation in the form of probiotics, can protect against allergic disease. For example, there is evidence that the protective effect of the farming environment on development of atopic sensitization, hay fever, and asthma is related to the wider range of microbial exposures in children living on farms compared to urban dwelling peers (Riedler et al., 200). This is supported by animal studies demonstrating that specific bacteria that are abundant in cowsheds, *Acinetobacter lwoffii* F78 and *Lactococcus lactis* G121, can attenuate allergic responses in mice (Debarry et al., 2007).

Clinical trials indicate that feeding mothers with *Lactobacillus rhamnosus* GG in the pre and early post-natal period may be effective in the treatment

and prevention of early atopic disease in children (Kalliomaki et al., 2001, 2003). In a similar vein, *Lactobacillus fermentum* was shown to be beneficial in improving the extent and severity of atopic dermatitis in young children (Weston et al., 2005). It should be noted that there have also been a number of clinical trials showing no effect of the same probiotic strains on the incidence or severity of allergic disease (Vliagoftis et al., 2008).

Animal models have provided strong evidence indicating that oral administration of certain microbes can have systemic effects on immune responses, it has been shown that perinatal treatment with *L. rhamnosus* GG suppresses the development of experimental allergic asthma in adult mice (Blumer et al., 2007; Feleszko et al., 2007). *L. rhamnosus* JB-1, could attenuate allergen induced allergic air-

way response in adult mice (Forsythe et al., 2007; Karimi et al., 2009) and others have since confirmed these anti-allergic effects in the airway using a variety of bacteria (Lim et al., 2009; Adam et al., 2010; Li et al., 2010). In addition, several strains of lactobacillus have been shown to attenuate allergic dermatitis when administered orally to mice (Inoue et al., 2007; Hacini-Rachinel et al., 2009; Won et al., 2011). A number of mechanisms have been identified that may contribute to the ability of these bacteria to attenuate allergic inflammation including altered antigen presentation by dendritic cells (Hart et al., 2004; Stagg et al., 2004), Th1 polarization (Adel-Patient et al., 2005; Hisbergues et al., 2007) or the induction of regulatory T cells (Karimi et al., 2009, 2012). More recently there has been evidence that certain Lactobacilli may influence the effector phase of adaptive inflammation (Schiffer et al., 2011). Specifically it is emerging that inhibition of mast cell responses is a component of the immunomodulatory effects of certain bacteria and may be a contributing factor to the ability of candidate probiotic organisms to attenuate allergic inflammation (Kim et al., 2008; Magerl et al., 2008; Oksaharju et al., 2011; Forsythe et al., 2012a).

The earliest evidence that commensal bacteria may communicate with mast cells came from the observations that non-pathogenic *E. coli* strains could inhibit mast cell activation following direct co-culture *in vitro* and *ex vivo* following injection of the bacteria into the peritoneal cavity of mice (Magerl et al., 2008). Wesolowski and Paumet (2014) recently provided some insight into the mechanism underlying the inhibitory effect of *E. coli* on mast cell degranulation. They determined that co-culture with *E. coli* disrupts the sequence of events leading to secretory granule fusion with the cell membrane.

Specifically the interaction between IKK β and SNAP23 is inhibited, resulting in the hypophosphorylation of SNAP23. Subsequent formation of the ternary SNARE complex between SNAP23, Syntaxin4 and VAMP8 is strongly reduced leading to impaired VAMP8-dependent granules release (Wesolowski and Paumet, 2014).

Lactobacilli have also been demonstrated to directly inhibit mast cell activation *in vitro*, however this ability appears to be highly strain specific (Kawahara, 2010; Oksaharju et al., 2011; Schiffer et al., 2011; Forsythe et al., 2012a). Overnight incubation with a strain of *L. casei* inhibits IgE-dependent mouse mast cell and human basophil activation (Schiffer et al., 2011). Inhibition could be induced by both viable and irradiated *L. casei*, suggesting the no involvement of secreted metabolites. A role for a structural component of the bacteria, rather than a secreted metabolite, is further supported by the observation that inhibition was also prevented when mouse mast cells were separated from bacteria by a semi-permeable membrane, indicating a requirement for *L. casei*/mast cell contact (Schiffer et al., 2011). However, the mechanism underlying the ability of lactobacilli to inhibit mast cell function is unknown and it may be that different strains employ distinct approaches to suppress cell activity. Indeed, *L. casei* induced inhibition of mast cells was not mediated by TLR or NOD1/2 receptors, while another study demonstrated that inhibition of IgE mediated mast cell activation by a strain of *L. reuteri* was at least partially mediated by TLR2 (Schiffer et al., 2011).

It is important to note that while evidence suggests that direct inhibition of mast cells by non-pathogenic bacteria *in vitro* requires cell contact with the microbe, many of these bacteria when delivered orally, or into the peritoneal

cavity, can inhibit systemic mast cell dependent responses. For example the requirement for direct bacteria mast cell interaction cannot readily explain the ability of *L. casei* to inhibit IgE mediated passive systemic anaphylaxis when delivered i.p. (Schiffer et al., 2011). Indeed evidence suggests systemic mast cell suppression may be mediated by a mechanism distinct for the effect of direct mast cell bacteria interaction.

The *Lactobacillus rhamnosus* strain JB-1 does not inhibit mast cell degranulation following direct *in vitro* co-culture (Forsythe et al., 2012a). However feeding JB-1 to rats inhibited peritoneal mast cell response to stimulation *ex vivo* and attenuates the passive cutaneous anaphylaxis response. These observations strongly suggest an indirect mechanism of action involving additional cell types (Forsythe et al., 2012a).

The exact nature of this mechanism and the potential intermediary cells are currently unknown. It has been reported that direct exposure of human peripheral blood mast cells to a *Lactobacillus rhamnosus* strain lead to a down-regulation of FcεR1 expression on the cell surface (Oksaharju et al., 2011). However, as treatment with JB-1 also inhibits degranulation in response to non-IgE mediated activation it is unlikely that changes in expression of this receptor account for the ob-

served inhibition of degranulation. It was demonstrated that feeding *Lactobacillus rhamnosus* was also associated with inhibition of IK_{Ca} current in peritoneal mast cells (Forsythe et al., 2012a). $KCa3.1$ channel current is critical to the function of many immune cells (Beeton et al., 2001; Wulff et al., 2003, 2004; Chandy et al., 2004) $KCa3.1$ opening is not required for, but potentiates, mast cell secretion (Duffy et al., 2004; Shumilina et al., 2008). The IK_{Ca} opener 1-EBIO enhances IgE-dependent Ca^{2+} influx and degranulation in response to a submaximal stimulus (Duffy et al., 2004), while mice from $KCa3.1$ deficient ($KCa3.1^{-/-}$) demonstrate attenuated degranulation in response to FcεR1 mediated activation (Shumilina et al., 2008). Indeed the degree of attenuation in response to IgE mediated activation of mast cells following *L. rhamnosus* feeding was similar to that observed in $KCa3.1$ deficient mice (Shumilina et al., 2008). The activation of a range of G_s -coupled receptors including β_2 -adrenoceptors, A_{2A} adenosine receptors and EP2 prostaglandin receptors can lead to inhibition of the IK_{Ca} current (Duffy et al., 2005, 2007, 2008). It is possible that other G_s -coupled receptors may also inhibit $KCa3.1$ opening and a variety of immune or neuronal derived mediators could be responsible for *L. rhamnosus* effects on the ion channel.

POTENTIAL MECHANISMS OF SYSTEMIC MAST CELL STABILIZATION BY BACTERIA

Galectins

The work of de Kivit et al. (2012) suggests galectins may play a role in the mechanism thorough which modulation of gut bacteria results in a systemic alteration in mast cell function. Galectins are secreted by keratinocytes, intestinal epithelial cells (and various

immune cells, including dendritic cells, macrophages and mast cells) (Hirashima et al., 2004; Rabinovich et al., 2009; Larsen et al., 2011; Smetana et al., 2013).

Galectins have been described to be involved in many physiological processes, including cell signalling, cell

adhesion, chemotaxis and cell apoptosis. In particular, Galectin-9 strongly and specifically binds IgE, a heavily glycosylated immunoglobulin, and that this interaction blocks IgE-antigen complex formation thus preventing degranulation. Interestingly Galectin-9 can be expressed by mast cells and immunological stimulation has been demonstrated to augment Galectin-9 secretion from the cells indicating that Galectin-9 is an autocrine regulator of mast cell function (Niki et al., 2009).

A diet containing prebiotic galacto- and fructo-oligosaccharides and a strain of *Bifidobacterium breve* protected against acute allergic symptoms and suppressed mast cell degranulation in whey-sensitized mice. The anti-allergic effects of the synbiotic treatment were correlated with increased galectin-9 expression by intestinal epithelial cells and increased levels of galectin-9 in serum (de Kivit et al., 2012). Galectin-9 is soluble-type lectin that recognizes β -galactoside containing glycans. Crucially, serum derived from whey-sensitized synbiotic-treated mice was able to suppress IgE-mediated mast cell degranulation and the extent of this suppression was correlated with serum galectin-9 (de Kivit et al., 2012). *In vitro* studies of mast cell degranulation involving RBL-2H3 cells demonstrated that Galectin-9 suppressed IgE mediated degranulation of the cells stimulated. This inhibitory effect was completely abrogated in the presence of lactose, indicating lectin activity of Galectin-9 is critical. Whether the increase in galectin-9 production applies universally to probiotic and prebiotic treatments that stabilize mast cells remains to be determined.

Quorum sensing molecules

In addition to inducing the release of signalling molecules in host cells bacteria themselves can produce an array

of molecules that can influence immune cell activity without the need for direct contact. Many species of bacteria communicate with each other through a cell density dependent signalling system, termed quorum-sensing (Walters and Sperandio, 2007; Kendall and Sperandio, 2007). These signals are mediated by small hormone-like molecules and allow a population of bacteria to coordinate their gene expression and activities. It is becoming evident that certain components of the quorum sensing system can also influence the behaviour of eukaryotic cells (Rumbaugh, 2007; Lowery et al., 2008). Many Gram-positive bacteria use small peptides to coordinate their activities (Kong et al., 2012). Gram-negative bacteria appear to use several types of small molecules. Of these the best-described signalling system is the N-acyl-homoserine lactone (AHSL) system. AHSLs are fatty-acid-based signalling molecules and mediate bacterial processes by interacting with inducible transcriptional regulators (Walters and Sperandio, 2006; Subramoni and Venturi, 2009). These molecules differ significantly only in the constituents of their fatty acid derived acyl chains. Homologs of the AHSL synthase (LuxI-type synthase) and AHSL receptor (LuxR-type receptor) regulate responses to the structurally distinct AHSL.

It has been demonstrated that AHSL, and in particular N-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL) which functions as a transcriptional regulator in *Pseudomonas aeruginosa*, can also alter mammalian cell responses. 3OC12-HSL can promote the up-regulation of pro-inflammatory cytokines and chemokines in lung epithelial cells induce apoptosis of neutrophils and macrophages and inhibit lymphocyte proliferation and TNF production (Ritchie et al., 2003,

2007; Vikström et al., 2006; Zimmermann et al., 2006). There have been somewhat contradictory reports regarding the effect of AHSL on mast cell function suggesting that 3OC12-HSL may attenuate mast cell responses by inducing apoptosis or enhance mast cell degranulation (Wu et al., 2001; Li et al., 2009). These differential effects may depend on the phenotype of the mast cell and the concentration of AHSL. Nevertheless, inter-kingdom communication, with bacteria derived signalling molecules influencing mast cell activity, is a potential means through which specific microbes may influence allergy development or disease severity and deserves further investigation.

Immunoregulation of Mast cells

There good evidence linking the immunomodulatory function of certain commensal bacteria, and components thereof, to induction of Treg and their associated cytokines (Feleszko et al., 2007; Karimi et al., 2009; Round and Mazmanian, 2010). Mazmanian et al. (2008) demonstrated that oral ingestion of polysaccharide A (PSA) derived from *Bacteroides fragilis* protects animals from experimental colitis through induction of IL-10-producing CD4⁺ T cells. *Bifidobacterium infantis* induces expression of Foxp3⁺ T cells that protect mice against *Salmonella typhimurium* infection (O'Mahony et al., 2008), while early life treatment of mice with *L. Rhamnosus GG* leads to an attenuated allergic airway response

in adult animals that is also associated with an increase in Foxp3⁺ T cells (Feleszko et al., 2007). *L. rhamnosus JB-1* significantly increases the proportion of CD4⁺CD25⁺Foxp3⁺ Treg cells in the mesenteric lymph nodes and spleen of non-sensitized adult mice (Karimi et al., 2009). In OVA-sensitized mice challenged with inhaled antigen this increase in Foxp3 was also observed in the mediastinal lymph nodes indicating that *L. rhamnosus* induced Treg can migrate to the airways in response to inflammation. Although the suppressive activity of Treg cells requires prior activation through their T-cell receptor, once activated, Treg cells can suppress in an antigen-non-specific way called “bystander suppression” (Tang et al., 2008). Recently, it has been shown that constitutive Foxp3⁺ Treg can control mast cell activation and IgE-dependent anaphylaxis in mice (Kanjarawi et al., 2013). Inhibition of mast cell degranulation by Tregs appears to require OX40/OX40 ligand interactions. It is known from *in vivo* transfer studies that a population of Treg cells can create a regulatory milieu that promotes the outgrowth of new populations of Treg cells with antigen specificities distinct from those of the original Treg population (Tang et al., 2008). It now appears that the stabilization of mast cells and reduction of mast cell dependent systemic responses following exposure to specific bacteria or shifts in microbiota composition may be a consequence of the T cell driven regulatory milieu.

MICROBIOTA-MAST CELL COMMUNICATION BEYOND ALLERGY

In addition to being at the frontline of host defence mast cells can translate signals between the nervous immune and endocrine systems (Theoharides, 1996; Forsythe et al., 2012b).

Considering effects beyond immunity, it should be noted that much of our earliest understanding of the relationship between the nervous and immune systems came from the study of mast

cell-nerve interactions. Indeed, mast cells have been described as neuro-immuno-endocrine master-players (*Theoharides*, 1996). Mast cells can be activated by a range of neurotransmitters and hormones while, in turn, a variety of molecules, including histamine and serotonin, synthesized and released by mast cells can influence neuronal activity and endocrine function (*Frieling et al.*, 1991, 1993). Similarly, mast cell derived cytokines including TNF and growth factors, such as NGF, modulate the threshold for activation of local neurons and promote nerve fibre growth (*Leon et al.*, 1994; *van Houwelingen et al.*, 2002; *Arnett et al.*, 2003; *Kakurai et al.*, 2006).

Mast cells have been demonstrated to influence behaviour of mice (with mast cell deficiency resulting in a more anxious phenotype) (*Nautiyal et al.*, 2008) and to participate in regulation of

the HPA axis (*Matsumoto et al.*, 2001). There is also strong evidence for mast cells as important participants in visceral hypersensitivity and pain perception, particularly in irritable bowel syndrome (*Barbara et al.*, 2007; *Wood*, 2007).

There are marked parallels between the neuroendocrine role of mast cells and the physiological effects described following exposure to non-pathogenic commensals/probiotic, (decreased intestinal permeability, myenteric nerve activation, anti-nociception, HPA regulation, and changes in anxiety-like behaviour) (*Kamiya et al.*, 2006; *Kunze et al.*, 2006; *Wang et al.*, 2010; *Bravo et al.*, 2011) and future studies designed to test potential causal relationships between neuroendocrine regulation and the ability of certain gut bacteria to modulate mast cell function will be of great interest.

LITERATURE

- Adam, E., Delbrassine, L., Bouillot, C., Reynders, V., Mailleux, A.C., Muraille, E., and Jacquet, A.: Probiotic *Escherichia coli* Nissle 1917 activates DC and prevents house dust mite allergy through a TLR4-dependent pathway. *Eur. J. Immunol.* 40, 1995-2005 (2010).
- Adel-Patient, K., Ah-Leung, S., Creminon, C., Nouaille, S., Chatel, J.M., Langella, P., and Wal, J.M.: Oral administration of recombinant *Lactococcus lactis* expressing bovine beta-lactoglobulin partially prevents mice from sensitization. *Clin. Exp. Allergy* 35, 539-546 (2005).
- Arnett, H.A., Wang, Y., Matsushima, G.K., Suzuki, K., and Ting, J.P.: Functional genomic analysis of remyelination reveals importance of inflammation in oligodendrocyte regeneration. *J. Neurosci.* 23, 9824-9832 (2003).
- Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., Cheng, G., Yamasaki, S., Saito, T., Ohba, Y., Taniguchi, T., Takeda, K., Hori, S., Ivanov, I.I., Umesaki, Y., Itoh, K., and Honda, K.: Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science* 331, 337-341 (2011).
- Barbara, G., Wang, B., Stanghellini, V., de Giorgio, R., Cremon, C., Di Nardo, G., Trevisani, M., Campi, B., Geppetti, P., Tonini, M., Bunnett, N.W., Grundy, D., and Corinaldesi, R.: Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. *Gastroenterology* 132, 26-37 (2007).
- Beeton, C., Wulff, H., Barbara, J., Clot-Faybessé, O., Pennington, M., Bernard, D., Cahalan, M.D., Chandy, K.G., and Beraud, E.: Selective blockade of T lymphocyte K(+) channels ameliorates experimental autoimmune encephalomyelitis, a model for multiple sclerosis. *Proc. Natl. Acad. Sci. USA* 98, 13942-13947 (2001).

- Blumer, N., Sel, S., Virna, S., Patrascan, C.C., Zimmermann, S., Herz, U., Renz, H., and Garn, H.: Perinatal maternal application of *Lactobacillus rhamnosus* GG suppresses allergic airway inflammation in mouse offspring. *Clin. Exp. Allergy* 37, 348-357 (2007).
- Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Bienenstock, J., and Cryan, J.F.: Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc. Natl. Acad. Sci. USA* 108, 16050-16055 (2011).
- Chandy, K.G., Wulff, H., Beeton, C., Pennington, M., Gutman, G.A., and Cahalan, M.D.: K⁺ channels as targets for specific immunomodulation. *Trends Pharmacol. Sci.* 25, 280-289 (2004).
- de Kivit, S., Saeland, E., Kraneveld, A.D., van de Kant, H.J., Schouten, B., van Esch, B.C., Knol, J., Sprikkelman, A.B., van der Aa, L.B., Knippels, L.M., Garssen, J., van Kooyk, Y., and Willemsen, L.E.: Galectin-9 induced by dietary synbiotics is involved in suppression of allergic symptoms in mice and humans. *Allergy* 67, 343-352 (2012).
- Debarry, J., Garn, H., Hanuszkiewicz, A., Dickgreber, N., Blumer, N., von Mutius, E., Bufe, A., Gatermann, S., Renz, H., Holst, O., and Heine H.: *Acinetobacter lwoffii* and *Lactococcus lactis* strains isolated from farm cowsheds possess strong allergy-protective properties. *J. Allergy Clin. Immunol.* 119, 1514-1521 (2007).
- Duffy, S.M., Berger, P., Cruse, G., Yang, W., Bolton, S.J., and Bradding, P.: The K⁺ channel iKCA1 potentiates Ca²⁺ influx and degranulation in human lung mast cells. *J. Allergy Clin. Immunol.* 114, 66-72 (2004).
- Duffy, S.M., Cruse, G., Lawley, W.J., and Bradding, P.: Beta2-adrenoceptor regulation of the K⁺ channel iKCA1 in human mast cells. *FASEB J.* 19, 1006-1008 (2005).
- Duffy, S.M., Cruse, G., Brightling, C.E., and Bradding, P.: Adenosine closes the K⁺ channel KCa3.1 in human lung mast cells and inhibits their migration via the adenosine A2A receptor. *Eur. J. Immunol.* 37, 1653-1662 (2007).
- Duffy, S.M., Cruse, G., Cockerill, S.L., Brightling, C.E., and Bradding, P.: Engagement of the EP2 prostanoid receptor closes the K⁺ channel KCa3.1 in human lung mast cells and attenuates their migration. *Eur. J. Immunol.* 38, 2548-2556 (2008).
- Feleszko, W., Jaworska, J., Rha, R.D., Steinhilber, S., Avagyan, A., Jaudszus, A., Ahrens, B., Groneberg, D.A., Wahn, U., and Hamelmann, E.: Probiotic-induced suppression of allergic sensitization and airway inflammation is associated with an increase of T regulatory-dependent mechanisms in a murine model of asthma. *Clin. Exp. Allergy* 37, 498-505 (2007).
- Forsythe, P., Inman, M.D., and Bienenstock, J.: Oral treatment with live *Lactobacillus reuteri* inhibits the allergic airway response in mice. *Am. J. Respir. Crit. Care Med.* 175, 561-569 (2007).
- Forsythe, P., Wang, B., Khambati, I., and Kunze, W.A.: Systemic effects of ingested *Lactobacillus rhamnosus*: inhibition of mast cell membrane potassium (IKCa) current and degranulation. *PLoS One* 7, e41234 (2012).
- Forsythe, P. and Bienenstock, J.: The mast cell-nerve functional unit: a key component of physiologic and pathophysiologic responses. *Chem. Immunol. Allergy* 98, 196-221 (2012).
- Frieling, T., Cooke, H.J., and Wood, J.D.: Serotonin receptors on submucous neurons in guinea pig colon. *Am. J. Physiol.* 261, G1017-G1023 (1991).
- Frieling, T., Cooke, H.J., and Wood, J.D.: Histamine receptors on submucous neurons in guinea pig colon. *Am. J. Physiol.* 264, G74-G80 (1993).
- Hacini-Rachinel, F., Gheit, H., Le Ludec, J.B., Dif, F., Nancey, S., and Kaiserlian, D.: Oral probiotic control skin inflammation by acting on both effector and regulatory T cells. *PLoS One* 4, e4903 (2009).
- Hart, A.L., Lammers, K., Brigidi, P., Vitali, B., Rizzello, F., Gionchetti, P., Campieri, M., Kamm, M.A., Knight, S.C., and Stagg, A.J.

- Modulation of human dendritic cell phenotype and function by probiotic bacteria. *Gut* 53, 1602-1609 (2004).
- Hirashima, M., Kashio, Y., Nishi, N., Yamachi, A., Imaizumi, T.A., Kageshita, T., Saita, N., and Nakamura, T.: Galectin-9 in physiological and pathological conditions. *Glycoconj. J.* 19, 593-600 (2004).
- Hisbergues, M., Magi, M., Rigaux, P., Steuve, J., Garcia, L., Goudercourt, D., Pot, B., Pestel, J., and Jacquet, A.: In vivo and in vitro immunomodulation of Der p 1 allergen-specific response by *Lactobacillus plantarum* bacteria. *Clin. Exp. Allergy* 37, 1286-1295 (2007).
- Inoue, R., Nishio, A., Fukushima, Y., and Ushida, K.: Oral treatment with probiotic *Lactobacillus johnsonii* NCC533 (La1) for a specific part of the weaning period prevents the development of atopic dermatitis induced after maturation in model mice, NC/Nga. *Br. J. Dermatol.* 156, 499-509 (2007).
- Kakurai, M., Monteforte, R., Suto, H., Tsai, M., Nakae, S., and Galli, S.J.: Mast cell-derived tumor necrosis factor can promote nerve fiber elongation in the skin during contact hypersensitivity in mice. *Am. J. Pathol.* 169, 1713-1721 (2006).
- Kalliomaki, M., Kirjavainen, P., Eerola, E., Kero, P., Salminen, S., and Isolauri, E.: Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J. Allergy Clin. Immunol.* 107, 129-134 (2001).
- Kalliomaki, M., Salminen, S., Poussa, T., Arvilommi, H., and Isolauri, E.: Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet* 361, 1869-1871 (2003).
- Kamiya, T., Wang, L., Forsythe, P., Goettsche, G., Mao, Y., Wang, Y., Tougas, G., and Bienenstock, J.: Inhibitory effects of *Lactobacillus reuteri* on visceral pain induced by colorectal distension in Sprague-Dawley rats. *Gut* 55, 191-196 (2006).
- Kanjarawi, R., Dy, M., Bardel, E., Sparwasser, T., Dubois, B., Mecheri, S., and Kaiserlian, D.: Regulatory CD4⁺Foxp3⁺ T cells control the severity of anaphylaxis. *PLoS One* 8, e69183 (2013).
- Karimi, K., Inman, M.D., Bienenstock, J., and Forsythe, P.: *Lactobacillus reuteri*-induced regulatory T cells protect against an allergic airway response in mice. *Am. J. Respir. Crit. Care Med.* 179, 186-193 (2009).
- Karimi, K., Kandiah, N., Chau, J., Bienenstock, J., and Forsythe, P.: A *Lactobacillus rhamnosus* strain induces a heme oxygenase dependent increase in Foxp3⁺ regulatory T cells. *PLoS One* 7, e47556 (2012).
- Kawahara, T.: Inhibitory effect of heat-killed *Lactobacillus* strain on immunoglobulin E-mediated degranulation and late-phase immune reactions of mouse bone marrow-derived mast cells. *Anim. Sci. J.* 81, 714-721 (2010).
- Kendall, M.M. and Sperandio, V.: Quorum sensing by enteric pathogens. *Curr. Opin. Gastroenterol.* 23, 10-15 (2007).
- Kim, J.Y., Choi, Y.O., and Ji, G.E.: Effect of oral probiotics (*Bifidobacterium lactis* AD011 and *Lactobacillus acidophilus* AD031) administration on ovalbumin-induced food allergy mouse model. *J. Microbiol. Biotechnol.* 18, 1393-1400 (2008).
- Kong, H.H., Oh, J., Deming, C., Conlan, S., Grice, E.A., Beatson, M.A., Nomicos, E., Polley, E.C., Komarow, H.D., Murray, P.R., Turner, M.L., and Segre, J.A.: Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res.* 22, 850-859 (2012).
- Kunze, W., Mao, Y., Forsythe, P., and Bienenstock, J.: Effect of *Lactobacillus* sp. (Lb) probiotic ingestion on membrane properties of intrinsic sensory neurons in rat colon. *Gastroenterology* 130, A401 (2006).
- Larsen, L., Chen, H.Y., Saegusa, J., and Liu, F.T.: Galectin-3 and the skin. *J. Dermatol. Sci.* 64, 85-91 (2011).
- Leon, A., Buriani, A., Dal Toso, R., Fabris, M., Romanello, S., Aloe, L., and Levi-Montalcini, R.: Mast cells synthesize, store, and release nerve growth factor. *Proc. Natl. Acad. Sci. USA* 91, 3739-3743 (1994).
- Leung, A.D., Schiltz, A.M., Hall, C.F., and

- Liu, A.H.: Severe atopic dermatitis is associated with a high burden of environmental *Staphylococcus aureus*. *Clin. Exp. Allergy* 38, 789-793 (2008).
- Li, C.Y., Lin, H.C., Hsueh, K.C., Wu, S.F., and Fang, S.H.: Oral administration of *Lactobacillus salivarius* inhibits the allergic airway response in mice. *Can. J. Microbiol.* 56:373-379 (2010).
- Li, H., Wang, L., Ye, L., Mao, Y., Xie, X., Xia, C., Chen, J., Lu, Z., and Song, J.: Influence of *Pseudomonas aeruginosa* quorum sensing signal molecule N-(3-oxododecanoyl) homoserine lactone on mast cells. *Med. Microbiol. Immunol.* 198, 113-121 (2009).
- Lim, L.H., Li, H.Y., Huang, C.H., Lee, B.W., Lee, Y.K., and Chua, K.Y.: The effects of heat-killed wild-type *Lactobacillus casei* Shirota on allergic immune responses in an allergy mouse model. *Int. Arch. Allergy Immunol.* 148, 297-304 (2009).
- Lowery, C.A., Dickerson, T.J., and Janda, K.D.: Interspecies and interkingdom communication mediated by bacterial quorum sensing. *Chem. Soc. Rev.* 37, 1337-1346 (2008).
- Magerl, M., Lammel, V., Siebenhaar, F., Zuberbier, T., Metz, M., and Maurer, M.: Non-pathogenic commensal *Escherichia coli* bacteria can inhibit degranulation of mast cells. *Exp. Dermatol.* 17, 427-435 (2008);
- Matsumoto, I., Inoue, Y., Shimada, T., and Aikawa, T.: Brain mast cells act as an immune gate to the hypothalamic-pituitary-adrenal axis in dogs. *J. Exp. Med.* 194, 71-78 (2001).
- Mazmanian, S.K., Round, J.L., and Kasper, D.L.: A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 453, 620-625 (2008).
- Moon, T.C., St. Laurent, C.D., Morris, K.E., Marcet, C., Yoshimura, T., Sekar, Y., and Befus, A.D.: Advances in mast cell biology: new understanding of heterogeneity and function. *Mucosal Immunol.* 3, 111-128 (2010).
- Nakamura, Y., Oscherwitz, J., Cease, K.B., Chan, S.M., Munoz-Planillo, R., Hasegawa, M., Villaruz, A.E., Cheung, G.Y., McGavin, M.J., Travers, J.B., Otto, M., Inohara, N., and Nunez, G.: *Staphylococcus delta-toxin* induces allergic skin disease by activating mast cells. *Nature* 503, 397-401 (2013).
- Nautiyal, K.M., Ribeiro, A.C., Pfaff, D.W., and Silver, R.: Brain mast cells link the immune system to anxiety-like behavior. *Proc. Natl. Acad. Sci. USA* 105, 18053-18057 (2008).
- Niki, T., Tsutsui, S., Hirose, S., Aradono, S., Sugimoto, Y., Takeshita, K., Nishi, N., and Hirashima, M.: Galectin-9 is a high affinity IgE-binding lectin with anti-allergic effect by blocking IgE-antigen complex formation. *J. Biol. Chem.* 284, 32344-32352 (2009).
- Noval Rivas, M., Burton, O.T., Wise, P., Zhang, Y.Q., Hobson, S.A., Garcia Lloret, M., Chehoud, C., Kuczynski, J., DeSantis, T., Warrington, J., Hyde, E.R., Petrosino, J.F., Gerber, G.K., Bry, L., Oettgen, H.C., Mazmanian, S.K., and Chatila, T.A.: A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. *J. Allergy Clin. Immunol.* 131, 201-212 (2013).
- Noverr, M.C. and Huffnagle, G.B.: The 'microflora hypothesis' of allergic diseases. *Clin. Exp. Allergy* 35, 1511-1520 (2005).
- Oksaharju, A., Kankainen, M., Kekkonen, R.A., Lindstedt, K.A., Kovanen, P.T., Korpela, R., and Miettinen, M.: Probiotic *Lactobacillus rhamnosus* downregulates FCER1 and HRH4 expression in human mast cells. *World J. Gastroenterol.* 17, 750-759 (2011).
- O'Mahony, C., Scully, P., O'Mahony, D., Murphy, S., O'Brien, F., Lyons, A., Sherlock, G., MacSharry, J., Kiely, B., Shanahan, F., and O'Mahony, L.: Commensal-induced regulatory T cells mediate protection against pathogen-stimulated NF-kappaB activation. *PLoS Pathog* 4, e1000112 (2008).
- Rabinovich, G.A. and Toscano, M.A.: Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation. *Nat. Rev. Immunol.* 9, 338-352 (2009).

- Riedler, J., Braun-Fahrlander, C., Eder, W., Schreuer, M., Waser, M., Maisch, S., Carr, D., Schierl, R., Nowak, D., and von Mutius, E.: Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 358:1129-1133 (2001).
- Ritchie, A.J., Yam, A.O., Tanabe, K.M., Rice, S.A., and Cooley, M.A.: Modification of in vivo and in vitro T- and B-cell-mediated immune responses by the *Pseudomonas aeruginosa* quorum-sensing molecule N-(3-oxododecanoyl)-L-homoserine lactone. *Infect. Immun.* 71, 4421-4431 (2003).
- Ritchie, A.J., Whittall, C., Lazenby, J.J., Chhabra, S.R., Pritchard, D.I., and Cooley, M.A.: The immunomodulatory *Pseudomonas aeruginosa* signalling molecule N-(3-oxododecanoyl)-L-homoserine lactone enters mammalian cells in an unregulated fashion. *Immunol. Cell Biol.* 85, 596-602 (2007).
- Round, J.L. and Mazmanian, S.K.: Inducible Foxp3⁺ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc. Natl. Acad. Sci. USA* 107, 12204-12209 (2010).
- Rumbaugh, K.P.: Convergence of hormones and autoinducers at the host/pathogen interface. *Anal. Bioanal. Chem.* 387:425-435 (2007).
- Schiffer, C., Lalanne, A.I., Cassard, L., Mancardi, D.A., Malbec, O., Bruhns, P., Dif, F., and Daeron, M.: A strain of *Lactobacillus casei* inhibits the effector phase of immune inflammation. *J. Immunol.* 187, 2646-2655 (2011).
- Shreiner, A., Huffnagle, G.B., and Noverr, M.C.: The "Microflora Hypothesis" of allergic disease. *Adv. Exp. Med. Biol.* 635, 113-134 (2008).
- Shumilina, E., Lam, R.S., Wolbing, F., Matzner, N., Zemtsova, I.M., Sobiesiak, M., Mahmud, H., Sausbier, U., Biedermann, T., Ruth, P., Sausbier, M., and Lang, F.: Blunted IgE-mediated activation of mast cells in mice lacking the Ca²⁺-activated K⁺ channel KCa3.1. *J. Immunol.* 180, 8040-8047 (2008).
- Smetana, K.J., Andre, S., Kaltner, H., Kopitz, J., and Gabius, H.J.: Context-dependent multifunctionality of galectin-1: a challenge for defining the lectin as therapeutic target. *Expert Opin. Ther. Targets* 17, 379-392 (2013).
- St. John, A.L. and Abraham, S.N.: Innate immunity and its regulation by mast cells. *J. Immunol.* 190:4458-4463 (2013).
- Stagg, A.J., Hart, A.L., Knight, S.C., and Kamm, M.A.: Microbial-gut interactions in health and disease. Interactions between dendritic cells and bacteria in the regulation of intestinal immunity. *Best Pract. Res. Clin. Gastroenterol.* 18, 255-270 (2004).
- Subramoni, S. and Venturi, V.: LuxR-family 'solos': bachelor sensors/regulators of signalling molecules. *Microbiology* 155, 1377-1385 (2009).
- Tang, Q. and Bluestone, J.A.: The Foxp3⁺ regulatory T cell: a jack of all trades, master of regulation. *Nat. Immunol.* 9, 239-244 (2008).
- Theoharides, T.C.: The mast cell: a neuroimmunoendocrine master player. *Int. J. Tissue React.* 18, 1-21 (1996).
- van Houwelingen, A.H., Kool, M., de Jager, S.C.A., Redegeld, F.A.M., van Heuven-Nolsen, D., Kraneveld, A.D., and Nijkamp, F.P.: Mast cell-derived TNF-alpha primes sensory nerve endings in a pulmonary hypersensitivity reaction. *J. Immunol.* 168, 5297-5302 (2002).
- Vikström, E., Tafazoli, F., and Magnusson, K.E.: *Pseudomonas aeruginosa* quorum sensing molecule N-(3 oxododecanoyl)-l-homoserine lactone disrupts epithelial barrier integrity of Caco-2 cells. *FEBS Lett.* 580, 6921-6928 (2006).
- Vliagoftis, H. and Befus, A.D.: Rapidly changing perspectives about mast cells at mucosal surfaces. *Immunol. Rev.* 206:190-203 (2005).
- Vliagoftis, H., Kouranos, V.D., Betsi, G.I., and Falagas, M.E.: Probiotics for the treatment of allergic rhinitis and asthma: systematic review of randomized controlled trials. *Ann. Allergy Asthma Immunol.* 101, 570-579 (2008).
- von Kockritz-Blickwede, M., Goldmann, O.,

- Thulin, P., Heinemann, K., Norrby-Teglund, A., Rohde, M., and Medina, E.: Phagocytosis-independent antimicrobial activity of mast cells by means of extracellular trap formation. *Blood* 111, 3070-3080 (2008).
- Walters, M. and Sperandio, V.: Quorum sensing in *Escherichia coli* and *Salmonella*. *Int. J. Med. Microbiol.* 296, 125-131 (2006).
- Wang, B., Mao, Y.K., Diorio, C., Wang, L., Huizinga, J.D., Bienenstock, J., and Kunze, W.: *Lactobacillus reuteri* ingestion and IK(Ca) channel blockade have similar effects on rat colon motility and myenteric neurones. *Neurogastroenterol. Motil.* 22, 98-107 (2010).
- Wesolowski, J. and Paumet, F.: *Escherichia coli* exposure inhibits exocytic SNARE-mediated membrane fusion in mast cells. *Traffic* 15, 516-530 (2014).
- Weston, S., Halbert, A., Richmond, P., and Prescott, S.L.: Effects of probiotics on atopic dermatitis: a randomised controlled trial. *Arch. Dis. Child.* 90, 892-897 (2005).
- Won, T.J., Kim, B., Lim, Y.T., Song, D.S., Park, S.Y., Park, E.S., Lee, D.I., and Hwang, K.W.: Oral administration of *Lactobacillus* strains from Kimchi inhibits atopic dermatitis in NC / Nga mice. *J. Appl. Microbiol.* 110, 1195-1202 (2011).
- Wood, J.D.: Neuropathophysiology of functional gastrointestinal disorders. *World J. Gastroenterol.* 13, 1313-1332 (2007).
- Wu, H., Song, Z., Givskov, M., Doring, G., Worlitzsch, D., Mathee, K., Rygaard, J., and Hoiby, N.: *Pseudomonas aeruginosa* mutations in *lasI* and *rhII* quorum sensing systems result in milder chronic lung infection. *Microbiology* 147, 1105-1113 (2001).
- Wulff, H., Beeton, C., and Chandy, K.G.: Potassium channels as therapeutic targets for autoimmune disorders. *Curr. Opin. Drug Discov. Devel.* 6, 640-647 (2003).
- Wulff, H., Knaus, H.G., Pennington, M., and Chandy, K.G.: K⁺ channel expression during B cell differentiation: implications for immunomodulation and autoimmunity. *J. Immunol.* 173, 776-786 (2004).
- Zimmermann, S., Wagner, C., Muller, W., Brenner-Weiss, G., Hug, F., Prior, B., Obst, U., and Hansch, G.M.: Induction of neutrophil chemotaxis by the quorum-sensing molecule N-(3-oxododecanoyl)-L-homoserine lactone. *Infect. Immun.* 74, 5687-5692 (2006).