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Skin microbiome and mast cells

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INTRODUCTION

Mast cells (MCs) are particularly frequent in the skin, airway and gut mucosa, where they are strategically located for optimal interaction with the environment and for their putative functions in host defense [1] and wound healing. They are regarded as major effector cells of immunoglobulin E (IgE)-mediated allergic inflammation. However, it has become clear that they also play important roles in both innate and adaptive immune responses. MCs possess a variety of surface receptors, including Toll-like and other microbe-associated pattern recognition receptors, and are activated by inflammatory mediators, IgE, IgG, light chains, complement fragments, proteases, hormones, neuropeptides, and microbial products [2, 3]. Following activation, they produce a plethora of pro-inflammatory mediators and participate in inflammatory reactions in many organs [2].

MCs have a hematopoietic origin and migrate as immature progenitor cells through the blood stream to peripheral tissues where they complete their maturation [4]. Stem cell factor (SCF) is essential for MC survival and development *in vitro* [5]. Other growth factors and cytokines can also influence maturation of MCs and contribute to determine the mature MC phenotype. Depending on the microenvironment they encounter, different subsets of mature MCs have been described [6–9].

An increased understanding of the importance of the microbiome to the normal physiology of mammals has led to a paradigm shift in immunology. The idea that the relationship between immunity and microbes is purely adversarial has been supplanted by an appreciation that the immune system has not only evolved to protect against pathogens, but also promotes and encourages a healthy microbiota. Under this paradigm, the role of MCs on the frontline of defense against pathogens suggests that they may also play an important role in fostering the host-microbiome relationship [3].

Although roles of MCs in the host-microbiome relationship have been well described, mainly in gut microbiomes [10–13], in this review we focus on the role of MCs in the skin

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and microbiomes of the skin, which have unique and different characteristics and functions from gut microbiomes [14, 15].

MAST CELL HETEROGENEITY

MC phenotypes are different, not only in different species, but also in different organs or tissues within the same species. In humans, the density of MCs has been described to be up to 500 to 4,000 cells/mm³ in the lungs, 7,000 to 12,000 cells/mm³ in the skin and 20,000 cells/mm³ in the gut [16]. The distribution of MCs in each organ is also strategic [17, 18]. For instance, in human skin, the densities of MCs tend to be higher in the forearms and lower legs, suggesting that regions that experience continual environmental exposure respond by either inducing more MC recruitment or proliferation in the local area [18].

A distinction of MCs established in mice is that connective tissue MCs (CTMCs) are constitutive and T cell-independent, while mucosal MCs (MMCs) are transient, induced in pathological settings, and T cell-dependent [4, 19]. CTMCs are detected mainly in the skin, peritoneal cavity, submucosal layer of the gut, and lung interstitium. Their granules contain heparin, as well as large amounts of histamine, and carboxypeptidase A3 (CPA3), and they can respond to cationic compounds (such as compound 48/80) by degranulating. In contrast, MMCs are usually found in the mucosal epithelium of the gut or lung. Their granules contain chondroitin, relatively lower amounts of histamine and CPA3, and show little or no response to cationic compounds [4, 19–21]. Although both MMCs and CTMCs express many kinds of serine proteases, including mouse MC proteases (mMCPs), their protease phenotypes are different depending on the location of the MCs [4, 22–25]. CTMCs in the skin and small intestine exhibit constantly stable expression of mMCP-4, 5, 6, 7, and CPA3, whereas in the small intestine, helminth-induced MMCs express only mMCP-1 [22, 25]. After seven daily challenges with aerosolized antigen to OVA-albumin sensitize BALB/c mice, both MMCs and CTMCs express mMCP-1, 2, 4, 5, 6, 7, and CPA3 in the trachea. In the large airway of the lung, CTMCs show the same expression pattern as in the trachea, but MMCs express only mMCP-1, 2, 6, and 7 [22] (Table 1).

In humans, MC subsets are usually classified into two types according to their protease content [21]. One subset of MCs contain only tryptase (MCT) and are mainly found in mucosal surfaces such as lung or gut epithelium (these are thought to resemble MMCs in rodents). The other subset of MCs contain tryptase, chymase, cathepsin G, and CPA (MCTC), and tend to reside for a long time within connective tissues (such as skin), submucosal tissues of the gut, and pulmonary perivascular tissues [17, 26] (these are thought to resemble CTMCs in rodents). The MCT/MCTC ratio is different in each tissue, but MCs that reside in skin are almost always MCTCs [17, 26] (Table 2).

While the classification of human MCs based on protease content is convenient, some studies have challenged these designations [29, 30]. For example, MCs containing chymase and CPA, but no tryptase (MC_C), exist [27, 28], but their location and function have yet to be elucidated. Furthermore, MCs infiltrating the airway epithelium in asthma express both tryptase and CPA, but not chymase [29], and MCs derived from adult peripheral blood progenitors express the full repertoire of MC proteases [30]. These data suggest that all MCs

arise from a common progenitor and that the local cytokine environment and cell-cell as well as cell-matrix interactions determine the levels of protease expression in MCs in each tissue type.

MAST CELL ACTIVATION

The skin has an important barrier function for exogenous hazards such as microbes, antigens, toxins, and physical stimuli. MCs support this barrier function and can act as sentinels in the skin. In MC activation, the most studied method is the allergic reaction, an adaptive immune response mediated by the high-affinity IgE receptor on the surface of MCs [31]. The role of MCs in innate immune regulation has also become important. MCs can recognize pathogens through different mechanisms including direct binding of pathogens or their components to pathogen-associated molecular pattern (PAMP) receptors on their cell surface [32]. Toll-like receptors (TLRs), Nod-like receptors (NLRs), retinoic acid-inducible gene 1 (RIG-I)-like receptors (RLRs), and receptors for complement cause most innate immune responses in MCs [33–36]. Once pathogens activate these receptors, inflammatory mediators are released. These mediators contribute to containing and clearing the infection and support adaptive immune responses, if necessary.

The expression patterns of PAMPs are different among MC subtypes in tissues. As summarized in Table 2, Kulka and Metcalfe showed some differences in TLR expression between *ex vivo* skin and lung MCs [37]. Motakis *et al.* also showed TLR expression in *ex vivo* skin and primary cultured skin MCs, but the expression patterns they observed were slightly different from those shown by Kulka and Metcalfe [37, 38]. Moreover, they showed that the expression levels of skin MC TLRs were much lower than those observed in other immune cells under stable conditions and that skin MCs can change their TLR expression levels when kept in culture [38]. McCurdy *et al.* also showed different expression patterns of TLRs in cord blood-derived mast cells (CBMCs) [39].

Recently, it has been proven that activation of TLR2, –3, –4, –6, –7, and –9 can induce degranulation and release of various cytokines by skin MCs [1, 34, 40, 41]. TLR2 recognizes peptidoglycans from gram-positive bacteria and mycobacteria. Supajatura *et al.* has also shown that *Staphylococcus aureus*-derived peptidoglycans can induce TLR2 during MC-dependent skin vasodilation and inflammation using the MC knock-in model [1]. TLR3 on the MC surface is the sensor for double-stranded RNA (dsRNA) and induces the production of antiviral cytokines, such as TNF- α and IFN- β , without MC degranulation [42]. Single-stranded viral RNA is sensed by TLR7 and TLR8, whereas viral DNA is detected by TLR9 [43]. MCs also express RIG-I protein kinase RNA-activated (PKR), and melanoma differentiation-associated gene 5 (MDA5), which recognize viral and synthetic dsRNA and induce MC activation and antiviral cytokine and chemokine production without degranulation [35, 36]. Human β -defensin-3 and 4 can also contribute to MC degranulation, prostaglandin D₂ generation, and chemotaxis [44].

BACTERIAL INFECTION

During bacterial infection, MCs facilitate bacterial clearance with their pathogen recognition ability and their release of mediators that induce increased vascular permeability and attract other immune cells [9]. MCs can also produce antimicrobial peptides (AMPs), such as cathelicidins, and have direct bactericidal activity [45, 46]. Cathelicidins in MCs are activated by TLR2 and TLR4 ligands [45, 47]. Moreover, the importance of cathelicidin activity for MC function has been demonstrated during group A *Streptococcus* skin infection; thus, not only cathelicidin derived from MCs kill bacteria directly, but also the presence of cathelicidin in MCs may act to facilitate recruitment of neutrophils [48] (Figure 1). It has also been demonstrated that MCs represent sentinel cells in the upper dermis, as their presence not only fights infection locally, but also prevents bacterial spread into the bloodstream [48]. On the other hand, when MCs are exposed to a very large number of bacteria, the dying MCs exert their extracellular antimicrobial activity against *Streptococcus pyogenes* through the production of extracellular structures called MC extracellular traps (MCETs), which are composed of DNA, histones, and granule proteins such as tryptase and cathelicidin LL-37 [49] (Figure 1).

VIRAL INFECTION

In viral infections, MCs are involved in the initiation of a chemokine-dependent host response [50–55] and release histamine in response to viral contact [50, 56, 57]. Aoki *et al.* showed the importance of MCs in host defense at herpes simplex virus (HSV)-2-infected sites through TNF- α and interleukin 6 (IL-6) production induced by keratinocyte-derived IL-33 [58]. In addition, Kurt-Jones *et al.* reported that TLR2 is a target for HSV-1 and that the TLR2-mediated cytokine response to HSV-1 is detrimental to the host [59]. Furthermore, Zhu *et al.* reported that innate immune recognition of vaccinia virus (VV) is mediated by TLR2; thus, *Tlr2*^{-/-} mice fail to produce proinflammatory cytokines after challenge with VV [60]. We have demonstrated that MCs degranulate upon VV fusion via interaction with the viral lipid envelope and that the response to VV in skin is substantially mediated by degranulation and cathelicidin expression in MCs [43]. This cathelicidin induction is caused by lipoteichoic acid (LTA), which is a TLR2 ligand [61] (Figure 2). Moreover, LTA-preconditioned wild-type MCs show increased AMP expression and greater antiviral response compared to pretreated *Tlr2*^{-/-} MCs. Blocking the release of granules with cromolyn abolished this response, confirming that LTA-induced AMP release is important. Furthermore, cathelicidin-deficient MCs could not improve their VV killing capacity after stimulation with LTA. These data also confirmed that LTA improved MC VV killing capacity through cathelicidin AMP [61]. This study revealed that LTA, released by bacteria at the skin surface, has the potential to be a potent mechanism to keep MCs vigilant at the port of entry of infections and to increase their capacity to fight viruses that use skin as an entry point.

SKIN INFLAMMATORY DISEASES

The skin of patients with atopic dermatitis (AD) shows greatly reduced microbial diversity relative to the same skin area of healthy controls. *Staphylococcus aureus* is found on the skin

in more than 90% of those affected with AD, though it is rarely present on healthy skin. Fortunately, there are some treatments to reduce *Staphylococcus aureus* colonization to decrease disease severity [62]. Moreover, it has been shown that the microbiome composition of lesional skin in children with moderate-to-severe cases of AD shifted temporally in association with disease flares and treatment [63]. Specifically, the relative abundance of *Staphylococcus aureus* and the skin commensal *Staphylococcus epidermidis* was increased with disease severity, while *Streptococcus*, *Propionibacterium*, and *Corynebacterium* genera were increased with treatment [63]. Peptidoglycans from *Staphylococcus aureus* have the ability to recruit MCs to the dermis through TGF- β produced by epidermal keratinocytes; thus, colonized *Staphylococcus aureus* in the AD skin surface might affect skin inflammation [64]. Nakamura *et al.* identified that δ -toxin, produced by *Staphylococcus aureus*, induces MC degranulation and that *Staphylococcus aureus* isolates recovered from patients with AD produce large amounts of δ -toxin. Furthermore, δ -toxin-induced MC degranulation was enhanced by IgE in the absence of antigen. In addition, skin colonization with *Staphylococcus aureus*, but not a mutant deficient in δ -toxin, increased IgE and IL-4 production and promoted inflammatory skin disease. Crucially, the enhancement of IgE production and dermatitis by δ -toxin was abrogated in MC-deficient *Kit^{W-sh/W-sh}* mice and restored by MC reconstitution [65]. This study identified that δ -toxin is a potent inducer of MC degranulation and suggested a mechanistic link between *Staphylococcus aureus* colonization and allergic skin disease.

P2 purinoceptors, P2X1 to P2X7, act as extracellular-ATP receptors [66]. Extracellular ATP stimulates MCs to release inflammatory cytokines, chemokines, and lipid mediators in a P2X7-dependent manner [67, 68]. Cathelicidin-derived peptide LL-37 can also directly stimulate P2X7 [69]. P2X7 is highly expressed on colonic MCs and P2X7-expressing MCs accumulate at lesional sites of Crohn's disease [67]. Conversely, skin MCs show low expression of P2X7 [67]. To maintain low expression of P2X7, skin fibroblasts produce the retinoic acid (RA)-degrading enzyme CYP26B1. Increased ATP production in RA-treated skin causes P2X7- and MC-dependent skin inflammation, known as retinoid dermatitis. Kurashima *et al.* reported that the skin inflammation induced by RA treatment was reduced in *Tlr2^{-/-}*, *MyD88^{-/-}*, and germ-free (GF) mice, and that cathelicidin expression was low in RA-induced skin inflammation lesions in GF mice. These data suggest that the skin microbiota induces TLR2-dependent production of P2X7 ligands, such as ATP and LL-37, from inflammatory monocytes and that such ligands activate MCs via P2X7. This pathway is critical for the development of retinoid dermatitis [70].

Rosacea is a chronic inflammatory skin disease. Frequent flares occurring without specific triggers harm many patients and, when left untreated, can take weeks to subside [71]. The formation of an aberrant AMP, cathelicidin LL-37, on the skin surface has been previously implicated as a key initiator of rosacea [72] and accompanies TLR2 overexpression on epidermal keratinocytes [73]. *Demodex* mites are present at a higher density in rosacea-affected skin [74], suggesting pathogenic association. We have demonstrated that cathelicidin LL-37, which is released from the epidermis in rosacea skin, activates skin MCs to release proteases such as matrix metalloproteinase (MMP) 9 and IL-6 [75]. These data were underscored by recent work showing that doxycycline, which is an effective therapeutic for improving rosacea symptoms, inhibits MMPs directly [76].

AUTOINFLAMMATORY DISEASES

Cryopyrin-associated periodic syndrome (CAPS) is a group of autoinflammatory syndromes caused by autosomal dominant mutations in the cryopyrin-coding gene *NLRP3* (Nod-like receptor, containing pyrin domain 3), also known as *CIAS1*, *PYPAF1* or *NALP3* [77]. Clinical manifestations, such as periodic fever and “urticaria-like rash”, that develop in the neonatal or early infant period are hallmarks of CAPS [78]. In addition, microbial components, such as lipopolysaccharide (LPS), peptidoglycan, and bacterial CpG DNA, are potent stimuli for initiating NLRP3 inflammasome activation [79]. All of these observations suggest that some environmental factors, even non-pathological bacterial colonization of the skin, may play a role in developing cutaneous manifestations. Indeed, Nakamura *et al.* revealed that the microbiome in the skin induces local production of TNF- α from MCs, which primes MCs themselves to elicit dysregulated production of IL-1 β , causing skin and systemic disease in neonatal mice with the CAPS-associated *Nlrp3* mutation [80].

THE MICROBIOME INFLUENCE

Changes in the microbiome have been connected with different skin diseases such as AD, acne, and psoriasis [81]. Specific commensal bacteria, such as *Staphylococcus epidermidis*, have been implicated in the modulation of skin responses to diseases [82] and recent studies suggest that the skin microbiota may play a functional role in immune cell modulation and differentiation [81]. However, little is known about whether or how the skin microbiome modulates the differentiation, survival, and function of MCs. Our group has recently published that the skin microbiome influences MC maturation in the dermis and that a normal microbiome is necessary for correct function and maturation of MCs [83]. In fact, in GF mice, MCs present lower expressions of the c-Kit and IgE receptors (Figure 3). Moreover, upon co-housing GF mice with specific pathogen-free (SPF) mice, GF mice regained their microbiome and converted back to a normal MC phenotype. Furthermore, when GF mice were stimulated *in vivo* with compound 48/80 injections in the skin, the resulting inflammation was much lower than in the SPF mice, suggesting altered MC function [83]. This discovery is of fundamental importance for a better understanding of the genesis of skin diseases like AD in which changes in the skin microbiome have been considered to be responsible for eczema flares.

CONCLUSION

Given the role of MCs as immune sentinels in the skin and mucosal surfaces, it is not surprising that these cells are involved in both defense against pathogens and in maintaining the beneficial relationship between the host and their microbiomes. In this review, we focused on the roles of the skin microbiome and MCs. The relationships between the two are wide-ranging, not only in preventing pathogens from invading through the skin, but also in modifying inflammation and associated disease pathogenesis. Improving our understanding of this relationship has the potential to create new strategies for treatment such as topical cromolyn for the lesions of rosacea [75].

Abbreviations

AD	atopic dermatitis
AMP	antimicrobial peptide
CAPS	cryopyrin-associated periodic syndrome
CPA	carboxypeptidase A
CTMC	connective tissue mast cell
dsRNA	double-stranded RNA
GF	germ-free
HSV	herpes simplex virus
Ig	immunoglobulin
IL	interleukin
LPS	lipopolysaccharide
LTA	lipoteichoic acid
MC	mast cell
MDA5	melanoma differentiation-associated gene 5
MMC	mucosal mast cell
mMCP	mouse mast cell protease
MMP	matrix metalloproteinase
NLR	nod-like receptor
NLRP3	nod-like receptor, containing pyrin domain 3
PAMP	pathogen-associated molecular pattern
PKR	protein kinase RNA-activated
RA	retinoic acid
RIG-I	retinoic acid-inducible gene 1
RLR	retinoic acid-inducible gene 1-like receptor
SCF	stem cell factor
TLR	Toll-like receptor
VV	vaccinia virus

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Figure 1. The two roles of mast cells (MCs) in bacterial infection

In group A *Streptococcus* skin infection, MC cathelicidin (LL-37) expression is increased by IL-4 and ligands of TLR2 and -4. The cathelicidins derived from MCs (LL-37) can not only kill bacteria directly, but also facilitate recruitment of neutrophils. On the other hand, MCs can show different antimicrobial activity against *Streptococcus pyogenes*, when they are exposed to a large number of bacteria, by emitting extracellular structures called MC extracellular traps (MCETs). IL-4: interleukin 4, LPS: lipopolysaccharide, LTA: lipoteichoic acid, TLR: Toll-like receptor

Figure 2. Skin microbiome-derived LTA reinforces the clearance of vaccinia virus (VV) with increasing antimicrobial peptide (AMP) expression in mast cells (MCs)

LTA derived from the commensal microbiome on the skin surface increases the expression of cathelicidins in MC granules via induction of *Cnlp*. TLR2 mediates this LTA-*Cnlp* response. VV challenge to MCs can also induce weak *Cnlp* expression in MCs, but this pathway is not mediated by TLR2. When VV enter MCs by membrane fusion, this event induces S1P formation and S1PR2 activation, leading to MC degranulation. *Cnlp*: cathelicidin-like protein, LTA: lipoteichoic acid, S1P: sphingosine-1-phosphate, S1PR2: S1P receptor 2, TLR: Tolllike receptor

Figure 3. Commensal skin microbiome promotes mast cell (MC) maturation

In a normal mouse, LTA from the skin microbiome induces keratinocytes to produce sufficient SCF, which results in proper MC maturation. In germ-free mice, MCs present lower expressions of the c-Kit and IgE receptors because SCF is insufficient. c-kit (CD117): mast/stem cell growth factor receptor, LTA: lipoteichoic acid, SCF: stem cell factor

Table 1

The differences in mature mast cell phenotypes in tissues

	Skin ^[22, 25]	Small intestine ^[22]	Lung ^[22]	Trachea ^[22]
MMC		mMCP-1	mMCP-1, 2, 6, 7	mMCP-1, 2, 4, 5, 6, 7, CPA3
CTMC	mMCP-4, 5, 6, 7, CPA3	mMCP-4, 5, 6, 7, CPA3	mMCP-1, 2, 4, 5, 6, 7, CPA3	mMCP-1, 2, 4, 5, 6, 7, CPA3

Table 2

The expression of TLRs in human mast cells

	TLR1	TLR2	TLR3	TLR4	TLR5	TLR6	TLR7	TLR8	TLR9	TLR10	
Skin ex vivo	-	+	+	+	+ low	-	+ low	-	+	+ low	Kulla <i>et al.</i> ^[37]
Skin ex vivo	+ low	+ low	+ low	+ low	ND	ND	+ low	ND	-	ND	Motakis <i>et al.</i> ^[38]
Skin cultured	+ low	+ low	+ low	+	ND	ND	+ low	ND	-	ND	Motakis <i>et al.</i> ^[38]
CBMC	+	+	ND	-	ND	+	ND	ND	ND	ND	McCurdy <i>et al.</i> ^[39]
Lung ex vivo	-	+	+	+	+ low	-	+	-	-	+ low	Kulla <i>et al.</i> ^[37]

CBMC: cord blood-derived mast cell, ND: not determined