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Mast cell activators as novel immune regulators

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Abstract

Mast cells are an important cell type of the innate immune system that when activated, play a crucial role in generating protective innate host responses after bacterial and viral infection. Additionally, activated mast cells influence lymph node composition to regulate the induction of adaptive immune responses. The recognition that mast cells play a beneficial role in host responses to microbial infection and induction of adaptive immunity has provided the rationale to evaluate mast cell activators for use as antimicrobials or vaccine adjuvants. This review summarizes the role of mast cell activators in antimicrobial responses while also discussing the use of different classes of mast cell activators as potent vaccine adjuvants that enhance the induction of protective immune responses.

Introduction

A long overlooked component of the body's immune arsenal are mast cells (MC), which are granulated hematopoietic cells that differentiate in the blood but mature in the tissues where they remain long lived, and sometimes capable of replicating when the tissue becomes overly inflamed. There are two major subsets of MC described in rodents, mucosal and connective tissue, that are distinguishable based on their granular contents. In recent years, several observations highlighting the ability of MCs to activate multiple arms of the immune system during infection have come to light. Through the release of immune suppressing mediators, MCs are also capable of contracting inflammation when it perceives that the infection is subsiding. In view of their powerful capacity to regulate the nature and intensity of inflammation at infection sites, there is a growing interest in the possibility of employing MC targeting immunomodulatory agents for medicinal use. Here we will review immunomodulatory roles of MCs during infection and some of the unique properties of MCs that support this role. We will also cover several studies demonstrating the efficacy of MC activators (MCAs) when used as adjuvants for vaccines. In view of the prior history of MCs

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as effectors of anaphylaxis, safety is an important consideration. We will discuss this topic as it pertains to the use of MCA as vaccine adjuvants.

Immunodulatory role of MCs

Since their discovery in 1863, MCs have mostly remained an enigma as their physiological role was difficult to pinpoint. For many years, interest in MCs was mostly linked to their prominent role in promoting various chronic inflammatory disorders such as asthma, allergic rhinitis, urticaria, inflammatory demyelinating disease and rheumatic disease[1–3]. The MC's role in these pathological conditions was primarily fostering excessive recruitment of various inflammatory cells into the inflamed site[4,5]. The first clues that MCs exhibit a beneficial and powerful role in combating various infectious diseases emerged only about two decades ago. These initial studies revealed that MCs at sites of infection have the innate capacity to directly recognize invading microbes or their products and promptly extrude their large collection of pre-stored granules [6,7]. MC granules contain various chemokines and cytokines as well as proteolytic agents and other agents which upon release, trigger the extravasation of various immune cells from the vasculature and the leakage of soluble vascular factors which together engage pathogens in infected tissue. The result of this MC mediated inflammatory response is accelerated clearance of the invading pathogen. For example, a mouse model of cytomegalovirus (CMV) infection demonstrated that CMV directly infects MCs and induces degranulation and secretion of CCL5[8], which recruits T cells to infection sites. MCs contribution to CMV clearance was shown using mice deficient in MC. In the absence of MCs, viral replication was significantly increased compared to animals with sufficient MCs. CMV also persisted in the lungs of MC deficient mice longer than wild-type mice. The role of MCs in CMV clearance was confirmed when MC deficient mice were reconsititued with MC and viral replication and clearance was similar to wild-type mice[8]. Concomitant with this vigorous innate immune response, MCs initiate the influx of different immune cells into lymph node that drain the infected site, which is the hallmark of the adaptive immune response [9,10]. Activation of draining lymph nodes (DLNs) occurs when MC granules released at the infection site reach this organ via the lymphatic system and release their cargo of pharmacologically active mediators. One of the most active MC granule compounds was TNF, since release of TNF from the MC granule triggers local production of key lymph node chemokines, such as CCL21, which induces massive migration of both antigen bearing dendritic cells from the site of infection and T cells from the circulation, causing significant lymph node hypertrophy[10]. The resulting interaction between incoming antigen presenting cells and recently sequestered T cells in the DLN resulted in a highly elevated pathogen-specific adaptive immune response. Thus, MCs have the capacity to initiate multiple arms of the immune system upon pathogen recognition at mucosal surfaces or the skin. However, not all MC responses to pathogens are beneficial. This is often the case after pathogens have overcome peripheral immune defenses and gained access into the blood where they now exhibit the capacity to simultaneously activate MCs all over the body. Under these circumstances, the wide-spread activation of MC leads to vascular leakage and shock.

The potent capacity of MCs to evoke protective and sometimes detrimental immune responses to pathogens is attributable to several unique traits. These include their

preponderance in the skin and mucosal sites, which is where most pathogens initiate infection of the host. MCs are readily activated at infection sites because they have receptors that directly bind a wide range of pathogens or for various host molecules that opsonize pathogens. MCs may also be activated at infection sites without direct contact with pathogens as they respond vigorously to danger signals (DAMPS) released by neighboring infected host cells[11]. In addition to releasing pre-stored inflammatory mediators, activated MC can de novo synthesize and secrete of a wide range of inflammatory mediator for several hours[12]. MCs not only initiate the inflammatory response but also sustain these reactions. Their capacity to regranulate and undergo multiple bouts of degranulation is also another but largely overlooked trait to prolong inflammation[13]. The MCs degranulation and mediator response to pathogens can be different depending on the pathogen. For example, MCs secrete proinflammatory cytokines but not type 1 interferon beta (IFN-β) when challenged by Gram positive and negative bacteria but MCs secrete anti-viral type 1 IFN when challenged by viruses[14]. This differential response of MCs may be attributable to the nature of the receptors on the MC surface engaged by each pathogen as the different surface receptors trigger distinct degranulation and mediator responses in MCs.

Mast Cell Activators as Antimicrobials

MCs possess antimicrobial properties and aide in killing bacteria and viruses[15]. Mouse models of infectious pathogens have demonstrated increased pathogen clearance in animals with sufficient MCs compared to mice that were deficient in MCs[16–18]. Activation of MC with toll-like receptor 2 (TLR2) ligands induces MC expression of cathelicidin, a class of host-defense peptides able to limit viral replication[17]. For example, maximal protective responses against vaccinia virus required TLR2 responsive MCs that produced antimicrobial peptides[16,17]. LL-37 is a cathelicidin peptide released from activated MCs that can directly kill microbes, such as *Streptococcus pneumoniae*[19]. In addition to being produced by MCs, LL-37 is also a MC activator[20,21]. LL37 induces MC migration[21], which may complement the antimicrobial properties of MCs since LL-37 may attract MCs towards infection sites to allow inflammatory mediators produced by MCs, such as Interleukin (IL)-1 and −8 and TNF and host defense peptides, contribute to host defense against bacteria and viruses[22]. Similarly, other peptides that activate MCs to degranulate, including retrocyclins (RC101) and protegrin-1, have antimicrobial properties and can kill *E.coli* in a dose-dependent manner[23].

In addition to activation by TLR ligands, MCs are activated by danger signals produced within the host after infection. Danger molecules, such as IL-33, which is released from viral-infected host cells, also activate MC to secrete the pro-inflammatory cytokine, TNFα and protect against a herpes simplex type 2 viral (HSV) infection[18]. In the absence of MCs or IL-33-responsive MCs, HSV-infected mice have significantly increased mortality compared to HSV-infected mice with WT MCs or MC deficient mice reconstituted with IL-33 responsive MC[18].This suggests that MCs are important for maximal protection against HSV infections. Taken together, the inflammatory responses resulting from MC activation appear to be beneficial in resolving microbial infections induced by bacteria and viruses; thus, it is reasonable to suspect that MC activators may be utilized therapeutically to enhance the antimicrobial activity of MCs and improve microbial clearance after infection.

Vaccine Adjuvant Activity of Mast Cell Activators (MCA)

In view of the powerful immunomodulatory capacity of MCs, particularly in boosting pathogen-specific adaptive immune responses during infection, the use of small molecule MC activators as adjuvants for vaccine is now being considered. For many decades, alum was the only adjuvant approved for use in FDA-approved vaccines. However, there has been a recent growth in the number of adjuvants used in FDA-approved vaccines, including the TLR4 ligand MPL and the TLR9 ligand CpG, due to the ability of the TLR ligands to provide adjuvant activity superior to that provided by alum. However, since TLR are present on many different types of host cells, these adjuvants have the potential to indiscriminately activate surrounding host cells making these inflammatory reactions difficult to regulate. In contrast, the MCAs most commonly studied as adjuvants are cationic molecules that target the MRGPRX2 G-protein-coupled receptor (MrgprB2 Is the mouse orthologue) that is found almost exclusively on MCs[24,25]. The best studied MRGPRX2 targeting MCA is Compound 48/80 (C48/80), a mixed polymer of p-methoxy-N-methyl phenylethylamine that induces extensive MC degranulation[26,27]. The vaccine adjuvant activity of C48/80 and other MC activators such as MC activating peptides, cytokines and nanoparticles will be discussed below.

C48/80.

The impetus for employing C48/80 as an adjuvant comes from the finding that instillation of this compound alone into the skin or nasal passages of mice was sufficient to trigger extensive degranulation of local MCs followed by simultaneous influx of dendritic cells and T cells into DLNs, just as if these peripheral sites were actively infected with bacteria [9,28]. When C48/80 was combined with recombinant protective antigen (rPA) from *Bacillus anthracis* and dermally injected into mice, high levels of antigen-specific antibody responses in serum and antigen-induced splenic cytokine responses was observed compared to the responses evoked by injection of the antigen alone[28]. The levels of the immune response induced by C48/80 was comparable to that evoked by cholera toxin or CpG, commonly utilized adjuvants enhancing Th2 or Th1 immune responses, respectively. The adjuvant effect of C48/80 was hypothesized to be associated with MC activation, which was deduced from the finding that most MCs at the site of dermal injection with C48/80, but not CT or CpG, appeared degranulated[28].

C48/80 also provides effective adjuvant activity with vaccines administered on mucosal surfaces. A potential benefit of mucosal immunization compared to parenteral immunization is the ability of mucosal immunization to induce adaptive immune responses in mucosal tissues, a common portal for pathogen entry. Nasal delivery of C48/80 with rPA significantly enhanced rPA-specific serum IgG responses even at very low doses of C48/80[29]. Importantly, in addition to the elevated serum levels of rPA-specific IgG antibodies, C48/80-adjuvanted intranasal rPA vaccines significantly enhanced rPA-specific IgA responses in salivary,vaginal and fecal secretions of immunized mice[29]. Thus MC targeting adjuvants applied to mucosal surfaces can potentially evoke immunity at other mucosal surfaces which is a useful way to protect against pathogens that typically infect via mucosal surfaces.

The potential of adjuvanted nasal vaccines to evoke protective mucosal immunity at various mucosal surfaces has inspired additional studies with MC activating C48/80 as a nasal vaccine adjuvant in other species. Since the nasal cavity in rabbits are anatomically more similar to that in man[30], rabbits are preferred to mice when nasal immunization is performed. Recently, immune responses to fragments of botulinum neurotoxin type A toxin or anthrax protective antigen in rabbits nasally instilled with and without C48/80 was compared[31,32]. Since cholera toxin (CT) represents the gold standard adjuvants for nasal vaccines, this adjuvant was also included for comparative purposes. In all cases, the rabbits immunized with C48/80-adjuvanted antigens evoked a much higher immune response compared to rabbits immunized with antigen alone. Indeed, the antibody responses induced by C48/80 were comparable to that induced by CT[31]. The C48/80-induced antibody response was functionally active and neutralized each of toxins[31,32]. This neutralizing power of the toxin specific antibody evoked by C48/80 correlated with high antibody avidity to the antigen[31]. Although C48/80 demonstrates effective adjuvant activity in mammalian models, it may not be effective in non-mammalian animal species. For example, C48/80 induces MC activation in gilthead seabream fish; however, when combined with killed bacteria antigens, C48/80 does not enhance humoral immunity compared to antigen alone[33]. Despite the ability of C48/80 to induce MC activation in fish species[34], it is possible that C48/80 is an ineffective adjuvant in fish species due to immunological differences between fish and mammals.

C48/80 has also been utilized as an effective nasal vaccine adjuvant when prepared as a dry powder vaccine formulation. In the vaccine studies mentioned, the formulation of vaccine antigen and C48/80 was in an aqueous phase. However, if the formulation could be dehydrated and delivered as a powder, it could be a huge advantage as the vaccine potentially could be stored at ambient temperature for long periods of time and avoid cold-chain storage and shipping, which are inconvenient and expensive. A dry powder C48/80-adjuvanted rPA nasal vaccine formulation was found to induce antibody responses comparable to the same vaccine in an aqueous formulation, indicating that spray freeze drying did not reduce the immunogenicity of the vaccine[32]. Additionally, powdered formulations maintained its capacity to induce rPA-specific serum IgG and anthrax lethal toxin-neutralizing antibodies even after 2 years of storage at ambient temperature. Additional studies have demonstrated that formulating C48/80-adjuvanted vaccines in nanoparticles further enhanced vaccine-induced immunity[35,36]. Thus, C48/80 provides effective adjuvant activity in liquid, powder or nanoparticle formulations and has the capacity to maintain adjuvant activity after ambient long-term vaccine storage.

Peptides.

Short peptides that activate MCs are attractive for use as vaccine adjuvants since they can be inexpensively and readily synthesized to high purity. MCs can be activated by peptides derived from endogenous and exogenous sources[37–39]. LL-37, which is found within leukocytes and several tissues and bodily fluids in humans[40], binds human MCs through the MRGPRX2 receptor triggering degranulation and de novo secretion of cytokines, such as IL-8[37]. The adjuvant activity of LL-37 was demonstrated using an oral vaccine against Dengue virus[41]. Oral delivery of LL-37 conjugated to the envelop domain III (EDIII)

antigen of Dengue virus significantly increased total and antigen-specific IgA responses in the fecal extracts of immunized mice compared to unimmunized mice (naïve) or mice immunized with EDIII alone[41]. Importantly, the antibody responses induced by LL-37-adjuvanted vaccines successfully neutralized Dengue virus in vitro demonstrating increased functionality compared to naïve mice or mice immunized with antigen alone[41].

Melittin is a 24 amino acid peptide found in bee venom with potent MC activating abilities and vaccine adjuvant activity. Nasal immunization of mice with this peptide combined with tetanus toxoid (TT) or diphtheria toxoid (DT) induced serum anti-TT and anti-DT IgG responses in a dose dependent fashion, compared to vaccination with antigen alone[42]. A concern with the use of relatively large exogenous peptides such as melittin is their capacity to evoke antibodies against themselves. In view of this concern, use of short peptides (<15 amino acid long) is preferable as they are less likely to be immunogenic.

Polymyxin B and colistin (polymyxin E) are two well-known antimicrobial drugs which are also potent MC activating peptides. Polymyxins have been reported to induce “rapid histamine release” from a variety of species, including humans, rats, hamsters, guinea pigs and mice[43,44]. The adjuvants properties of polymyxin B and colistin were observed in mice using ovalbumin (OVA) as antigen. Nasal immunization with polymyxin B or colistin increased antigen-specific mucosal IgA responses in a dose-dependent manner in the feces, nasal secretions and saliva and antigen-specific IgG and IgA responses in the serum. That polymyxin B was found to evoke an adjuvant-specific IgM response against itself but not an adjuvant-specific IgG response[39] could limit its consideration as an adjuvants . In view of the existence of several other small MC activating peptides, this class of MC activators has a high chance of being incorporated in human vaccines.

Cytokines.

The correlation of MC activation with vaccine adjuvant activity has increased the interest of MC activating molecules as vaccine adjuvants. Interleukin (IL)-1 family cytokines have previously been described as vaccine adjuvants when delivered intranasally[45]. IL-18 and IL-33 are members of the IL-1 family cytokines that are activate MC. IL-18 activates MCs by binding the IL-18Rα in the presence of IL-3 to increase histamine and T helper type 2 (Th2) cytokines, IL-4, IL-13[46]. IL-33 utilizes IL1RL1 (ST2) receptor to activate MCs[47]. IL-33-induced MC activation does not directly induce degranulation but increases MCs to release pro-inflammatory cytokines, including IL-4, −5, −6, −8, −13 and TNFα[48]. However, if MC degranulation is induced by FcεR1 crosslinking, then IL-33 can enhance degranulation-induced inflammation. Nasal immunization with recombinant influenza hemagglutinin (rHA) combined with MC activating cytokines IL-18 and IL-33 demonstrated significantly enhanced rHA-specific antibodies in the serum and mucosal secretions of mice compared to those immunized with antigen alone[45]. IL-33-adjuvanted rHA vaccines induced 80% survival from a lethal influenza challenge; whereas, IL-18-adjuvanted rHA vaccines induced 100% protection. While both IL-18 and IL-33 activate MCs, IL-18 (but not IL-33) required MCs since MC deficient mice developed antigen-specific adaptive immune responses lower than wild-type mice after nasal immunization with IL-18-adjuvanted vaccines[45]. Endogenous cytokines are an interesting source of MC activators since they

are host derived but may not be practical for vaccine development due to the high cost to produce recombinant cytokines.

Safety considerations.

As with any therapeutic to be used in humans, it is important to determine if the therapeutic is safe. Due to their role in mediating IgE-mediated allergic responses, it is important to determine if the use of MCA as antimicrobials or vaccine adjuvants exhibits any detrimental adverse effects, including the induction of IgE responses, which may contribute to an anaphylactic response. Intradermal delivery of C48/80 induced antigen-specific IgE responses similar to those induced by immunization with antigen alone[28], suggesting that the inclusion of C48/80 as an adjuvant in an intradermal vaccine is safe and did not enhance IgE responses. However, repeated delivery of C48/80 at a high adjuvant dose delivered nasally did induce elevated antigen-specific IgE responses in the serum and local mucosal secretions[49], suggesting that MCA have the potential to induce adverse immune responses with prolonged use. Additional studies are needed to thoroughly evaluate the safety of MCAs in order to justify their development for use in humans. Although MCA may contribute to enhanced host inflammation and may induce off-target effects maintaining localized MC activation through proper formulation may reduce potential adverse events and increase safety profiles.

Conclusions

MCs are an important cell of the host innate immune system that contribute to the host response to infectious agents while also contributing to the induction of adaptive immune responses (Figure 1). Compounds able to activate MCs may therefore be developed as therapeutics to treat microbial infections or as vaccine adjuvants to enhance the induction of protective immune responses. Several published studies now demonstrate the ability of different classes of MCAs to provide potent and safe vaccine adjuvant activity. Additional studies in rodents and other preclinical models are needed to better define the use of MCAs as antimicrobials and vaccine adjuvants and to evaluate their safety.

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Highlights

- Mast cells are innate immune cells that possess immunoregulatory activity.
- Mast cells exhibit antimicrobial activity and aide in bacterial and viral clearance.
- Mast cell activators enhance host antimicrobial activity.
- Mast cell activators provide vaccine adjuvant activity.

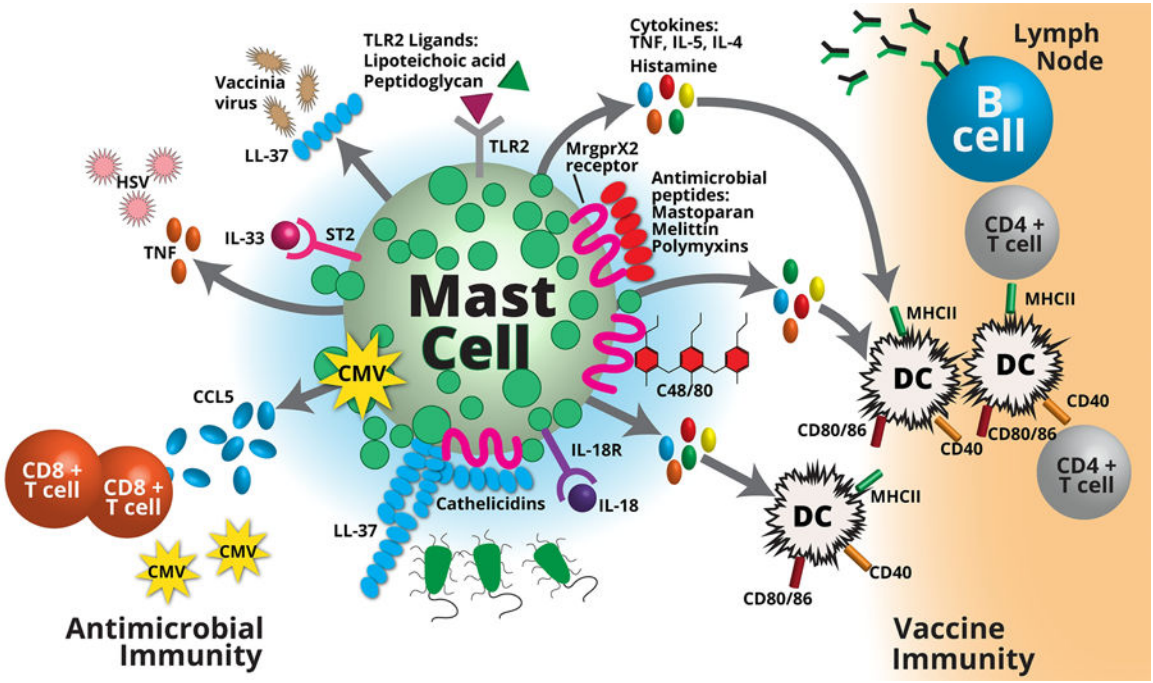

Figure 1. Mast cell activators contribute to host immunity in response to vaccines and antimicrobial infection.

Mast cell activators can be derived from endogenous sources, including the cytokines, IL-18 and IL-33 and host defense peptides, such as LL-37, as well as exogenous molecules, including microbial byproducts that activate host pattern recognition receptors and certain antimicrobial peptides and small molecules that bind the MrgprX2 receptor. Mast cell activation can result in immediate degranulation and release of various inflammatory mediators pre-stored in mast cell granules (green circles) which is followed by de novo synthesis and secretion of an array of biologically active mediators. Several of these discharged mast cell mediators, including cytokines, such as TNF, IL-1, IL-4 and IL-5, chemokines, such as CCL5, histamine and antimicrobial peptides aid in recruiting immune cells to the site of infection to clear pathogens. Similarly, mast cell mediators promote the infux of various immune cells including antigen presenting cells (APCs) to the site of immunization. This mast cell induced inflammatory reaction also enhances APC maturation trafficking to the draining lymph nodes (DLNs). Mast cell granules following exteriorization, traffic to the DLNs via the lymphatics where they release their cargo of inflammatory mediators which in turn induce the production of chemoattractants. These lymph node chemottractants mediate the simultaneous recruitment of APCs from the inoculation site and T cells from the circulation into the DLNs. The result of these mast cell induced trafficking into the DLN is enhanced adaptive immune response to the vaccine antigen. Thus, molecules that activate mast cells can induce inflammatory responses to enhance vaccine-specific and antimicrobial immunity.