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Review Article

Roles and relevance of mast cells in infection and vaccination

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Abstract

In addition to their well-established role in allergy mast cells have been described as contributing to functional regulation of both innate and adaptive immune responses in host defense. Mast cells are of hematopoietic origin but typically complete their differentiation in tissues where they express immune regulatory functions by releasing diverse mediators and cytokines. Mast cells are abundant at mucosal tissues which are portals of entry for common infectious agents in addition to allergens. Here, we review the current understanding of the participation of mast cells in defense against infection. We also discuss possibilities of exploiting mast cell activation to provide adequate adjuvant activity that is needed in high-quality vaccination against infectious diseases.

Keywords: mast cell, infection, host defense, vaccine, adjuvant

Introduction

Classically mast cells are considered critical effector cells in allergy by virtue of their potential to secrete a variety of allergic mediators. The number of mast cells is increased at sites of allergic inflammation, and there is a correlation between mast cell density in the tissue and the severity of allergic symptoms[1]. In allergy, plurivalent antigens bind and crosslink IgE molecules bound to the high-affinity IgE-receptor (FcεRI) expressed on mast cells, resulting in cell degranulation and release of proinflammatory mediators. Three major categories of mast cell mediators have been described: (1) preformed granule-associated mediators such as histamine and serotonin; (2) newly generated lipid mediators such as leukotrienes and prostaglandins; (3) de novo synthesized cytokines including chemokines. IgE-mediated activation of mast cells initiates the early phase of allergic responses, resulting in pathologies including greater epithelial permeability, mucus production, smooth muscle contraction, vasodilitation and neurogenic inflammation. The immediate response is followed by recruitment of a variety of other immune cells that participate in the late phase of the reaction, further exacerbating allergic pathology[1].

Mast cells are derived from hematopoietic progenitors in the bone marrow which migrate via blood to tissues all over the body where they further differentiate and mature into different phenotypes, depending on the local microenvironment. Stem cell factor (SCF), also known as steel factor, KIT ligand, or mast cell growth factor, is found to be the primary growth and differentiation factor for mast cells[2]. The cellular receptor for SCF is the product of the c-*kit* proto-oncogene. In addi-

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tion to SCF, mast cell growth and differentiation can be facilitated by several other cytokines including IL-3. For example, expansion of tissue mast cells upon nematode infection requires IL-3[3-4]. Immature mouse mast cells can be differentiated *in vitro* from bone marrow precursor cells in the presence of IL-3 without SCF[5]. Mast cells are enriched in the skin, around blood vessels, and in mucosal membranes such as the respiratory and gastrointestinal tracts. Most notably, mast cells are highly enriched in the skin and mucosal barriers of the body, where they serve as a first line of defense. It is noteworthy that mature mast cells are capable of differentiating both phenotypically and functionally as a consequence of tissue-specific stimulation under defined microenvironmental conditions. For example, inflamed lungs are reported to have more tryptase/chymase-producing mast cells compared with non-inflamed lung tissue in which tryptase-producing mast cells are dominant[6-7].

Mast cell subtypes

Two major subtypes of rodent mast cells have been characterized, i.e. connective tissue mast cells (CTMC) and mucosal mast cells (MMC), based on their tissue localization[8-11]. For instance, skin mast cells and mast cells residing in the peritoneal cavity are CTMC, whereas mast cells located in the respiratory or gastrointestinal tracts are usually characterized as MMC. In addition to tissue localization, other properties such as protease and cytokine profiles, membrane receptor distribution, and growth factor requirements also distinguish these two types of mast cells. In addition to residing in connective and serosal tissues, CTMC in mice have been found in the submucosa of the stomach[12] and nasal tissue[13].

In contrast, human mast cells are usually grouped based on the expression pattern of two mast cell-specific proteases, i.e. tryptase and chymase. According to this classification, two major human mast cell subgroups have been proposed. Mast cells that contain only tryptase are referred to as MC_T, whereas those that contain both tryptase and chymase are termed MC_{TC}. In terms of correlation to their murine counterparts, MC_T are found mainly in mucosal tissues, resembling mouse MMC, while MC_{TC}, which reside in such sites as the skin and small intestinal submucosa, are more closely related to mouse CTMC[14], although the tissue localization is less stringent for human "CTMC" and "MMC". Similar to mouse mast cells, human mast cells also differ in the requirement for growth and differentiation factors. Specifically, SCF is needed for the survival of both types, whereas IL-4 is indispensable for MC_{TC}, but not for MC_T[15].

Multitalented cells beyond allergy

In addition to IgE- and FcεRI-mediated cell activation, mast cells can be activated by a variety of other stimulators, such as IgG immune complexes, cytokines, complement components, neuropeptides, chemical agents, and physical stimuli, as mast cells express broad-ranging surface receptors including Fc receptors, complement receptors, and pathogen-associated molecular patterns (PAMP) such as Toll-like receptors (TLR). These observations, together with the description of a wide spectrum of mast cell mediators, provide a basis for proposals implicating mast cells in almost all aspects of immune responses. Therefore, mast cells have been postulated to be modulators of numerous physiological and pathological responses beyond their classically defined role in allergies mediated mainly through FcεRI. These multifunctional properties of mast cells have been more extensively reviewed elsewhere[16-17]. It has to be pointed out that the overwhelming research findings addressing the roles of mast cells have relied on the use of mast cell-deficient, KIT mutant mice which have other phenotypic abnormalities in addition to mast cell deficiency. These data await further experimental verification using the KIT-independent mast cell-deficient models to eliminate the confounding elements as a result of KIT mutation[18].

The roles of mast cells in host defense

The earliest observation of a beneficial role of mast cells is their potential in defense against parasitic infection[19-20]. The MMC pool expands extensively during nematode infection, a process dependent on IL-3[3-4]. Both IgE and mouse mast cell protease-6 (mMCP-6) are required for chronic immune responses against *Trichinella spiralis* infections[21]. In a helminth infection model, mast cells contribute to pathogen clearance by migrating to the draining lymph nodes and producing IL-6 and IL-4[22]. Interestingly, mast cells have also been described to be critical for Th1 response-mediated defence against oral infection with *Toxoplasma gondii*[23]. In addition to defense against helminth infections, mast cells have also been described to be protective in bacterial infections. One of the classic examples of mast cell-dependent anti-bacterial infection is demonstrated by the cecal ligation and puncture (CLP) model of acute peritonitis which is dependent on tumour necrosis factor (TNF)[24] and the ability of mast cells to lower neurotensin levels[25]. Mast cells harbour antimicrobial peptides including cathelicidin in their secretory granules[26]. Furthermore, β-hexosaminidase, which is abundantly contained in mast cell granules, has recently been reported to have bactericidal activity[27]. The roles and

relevance of mast cells in defense against viral and fungal infections have also been suggested[28-29].

Pathogen-mediated mast cell activation can be achieved through several mechanisms. Mast cells can be activated, through the equipped TLR, by direct recognition of microbial components such as bacterial lipopolysaccharide (LPS) and peptidoglycan resulting in distinct outcomes[30-32]. Mast cells can respond to microbial stimuli by surface proteins such as CD48[33-34]. Furthermore, mast cells can be stimulated by endogenous inflammatory factors such as cytokines and complement components secondary to infection[35-36]. Indirect interaction of mast cells with pathogens can also be achieved through the recognition of pathogen-antibody complexes by Fc receptors including FcεRI and Fcγ receptors expressed on mast cells[37-39]. Fc receptor-mediated mast cell activation may also be triggered in the presence of certain pathogen-derived proteins that can bind immunoglobulins in an antigen-independent manner. A classic example of such a bacteria-derived superantigen is protein A from *Staphylococcus aureus* which can activate human and mouse tissue mast cells[40-42], as the FcεRI molecules on these mast cells are most likely to have already been occupied with IgE, resulting in crosslinking of FcεRI upon protein A binding. However, the pathophysiological roles of such superantigen-mediated mast cell activation in defence against infection await further clarification.

Similar to mast cell activation in other circumstances, the activation by pathogens is also believed to include both degranulation of pre-formed granular contents and selective *de novo* mediator production, for example, cytokines and lipid mediators, the patterns of which differ greatly depending on the stimulus encountered. These mast cell-associated products, such as TNF, IL-4, OX40 ligand and mMCP-6, are important for the recruitment and stimulation of other innate immune participants, *e.g.* neutrophils, macrophages, natural killer (NK) cells and eosinophils, contributing to the clearance of pathogens[21,30,43-44]. Mast cells not only interact with cells in the immediate vicinity where the infection first takes place but also influence distant targets, *e.g.* cells in lymph nodes through mediators that they release[45]. It is also reported that mast cells can kill bacteria by producing extracellular traps that contain antimicrobial mediators[46].

In addition to contributing to innate immune responses by virtue of their large spectrum of granular products, mast cells also form a link between innate and adaptive immunity. Mast cells modulate the phenotype and function of key players in adaptive immunity, such as dendritic cells (DC), B cells, and T cells. Mast cells have been shown to functionally interact with professional antigen presenting cells (APC) such as DC and regulate their function mainly through mast cell-derived granular products. For example, histamine is capable of regulating the chemotaxis of immature DC[47-48] and cross-presentation of extracellular antigens[49]. TNF produced from mast cells is critical for DC migration[50-52]. TLR7 ligand-mediated mast cell activation is effective for the migration and maturation of Langerhans cells[53]. Maturation and activation of immature DC by mast cell-DC direct contact results in the activation of T cells that release IFN-γ and IL-17 promoting Th1 and Th17 responses, respectively[54]. Mast cells provide essential signals such as IL-6 and CD40L to enhance proliferation of B cells and drive their differentiation toward IgA-secreting plasma cells[55]. Mast cells can enhance the activation of T cells by providing costimulatory signals and secreting TNF[56-58]. Mast cells also contribute to the recruitment of T cells to sites of viral infection by secreting chemotactic molecules[59-60].

One of the key processes in achieving successful adaptive immunity is the presentation of microbial antigens to T lymphocytes. Whether or not mast cells are capable of acting as antigen-presenting cells is still controversial[61-65]. This is largely because of the argument that mast cells under steady state do not seem to constitutively express major histocompatibility complex class II (MHC-II) or co-stimulatory molecules such as CD86[63-64]. In contrast, mast cells upregulate expression of MHC-II and costimulatory molecules following stimulation by inflammatory factors such as IFN-γ and LPS[63-64]. Therefore, mast cells may have the potential to directly present antigens to T cells at least under certain circumstances, for example, in inflamed tissues, to initiate adaptive immune responses. Mast cells have also been demonstrated to present antigen to and activate $CD8^+$ T cells through MHC-I molecules[66-67].

Alternatively, mast cells are reported as participating in antigen cross-presentation to T cells[68]. Cross-presentation refers to a process, most typically following intracellular microbial infection, during which professional APC ingest infected cells and display the antigens of the microbes originally engulfed by the infected cells for recognition by T lymphocytes[69]. This is an efficient mechanism for presenting the antigens of those microbes that have infected host cells that may not produce all the signals, *e.g.* MHC-II recognition and costimulation needed to initiate T cell activation. The professional APC that have ingested infected cells may present the microbial antigens to both $CD4^+$ and $CD8^+$ T lymphocytes depending on the processing and presentation routes. Morphological changes of the host cells as a result of, *e.g.* microbial infections, apoptosis, and tumourigenesis, will facilitate ingestion by APC. In principle, any type of cells that have internalized antigens can participate in cross-presentation upon ingestion by APC. Importantly, mast cells have been implicated in the

phagocytosis of various types of antigens[70-73]. Various mechanisms have been reported for mast cells to internalize bacterial pathogens[74-76]. Indeed, mast cells can serve as an antigen-reservoir and participate in antigen cross-presentation[68]. *In vitro* cultured bone marrow-derived cultured mast cells (BMMC) can internalize IgE-bound chicken ovalbumin (OVA) protein, followed by engulfment by DC which process and present the OVA peptide to T cells that have specific receptors for the OVA peptide[68]. Induction of BMMC apoptosis is documented to be critical for efficient presentation by DC to T cells of the antigen originally phagocytosed by mast cells[68].

Owing to the fact that mast cells are capable of participating in both innate and adaptive immunity, and that they are enriched at the mucosal and skin barriers between the body and the external environment, mast cells, similar to skin Langerhans cells, tissue-resident DC and epithelial cells, are believed to be sentinel cells that are probably the first responders to a threat within seconds. Equipped with their immunologic armory of mediators, mast cells may possibly exert a pivotal role in the surveillance and elimination of pathogens by diversified mechanisms.

While people have been endowing mast cells with a more positive image in health, new findings also implicate mast cells or their released products negatively in infection. Although mast cell-associated TNF has been reported to be critical for a CLP model of acute peritonitis[24], it has to be pointed out that mast cell-derived TNF is not always protective in acute peritonitis, especially in models of severe CLP[77]. The detrimental effects of mast cells in severe peritonitis have also been ascribed to the release of IL-4 that inhibits the phagocytic potential of macrophages[78]. Mast cell degranulation may contribute to vascular leakage that may exacerbate dengue virus infection[38]. Even the potential of mast cells to recruit other immune effector cells during an infection is not always protective as this has been found to promote *Chlamydia pneumoniae* infection[79]. Interestingly, mMCP-4, the mouse counterpart of human mast cell chymase, can degrade TNF, thus dampening the severity of inflammation associated with sepsis and limiting the damage caused by TNF[80], suggesting antagonism between mast cell mediators, thus favouring protection. Therefore, the implication and relevance of mast cells in host defense is a complex issue and the net outcome may depend on many antagonistic factors.

The implication of mast cells in vaccination

A vaccine is a biological preparation that stimulates an immune response against specific antigens that either are derived from the pathogen itself or resemble the structure of the pathogen. Ever since the first documented vaccination attempt by Edward Jenner for the prevention of small pox in 1796, vaccines have played a crucial role in protecting people against many infectious diseases[81-82]. The eradication of smallpox and the effective control of polio represent two classic success stories of how vaccines can play a major role in improving global health. Nevertheless, the demand for better and more effective vaccines against many infectious diseases is still growing, especially when infections such as tuberculosis, HIV, dengue fever and malaria still present enormous global problems. From a societal point of view, vaccination remains the most effective intervention in the control of infectious diseases and for the improvement of global health.

There are two principal forms of vaccines: those that are live attenuated vaccines and those that are killed whole pathogens or subcomponent vaccines. An advantage of live attenuated vaccines is that they usually stimulate long-term immune responses similar to natural infection. However, live attenuated vaccines always come with a risk of reversion into more virulent organisms that could cause adverse reactions or more severe infections. In contrast, killed vaccines or subcomponent vaccines are more predictable and, therefore, safer. Furthermore, another concern that makes live attenuated vaccines less practical is the demand for a cold-chain for storing or transporting these vaccines. Therefore, killed vaccines are still much in use, even though they are weaker and usually do not promote as strong long-term memory responses.

To make killed vaccines more effective, we need adjuvants which are substances that enhance immune responses and stimulate long-lasting, robust protective immunity. An adjuvant that is included in the vaccine contributes greatly to the efficacy of the vaccination by affecting the immune responses both quantitatively and qualitatively. Importantly, protective immunity following vaccination may be generated with lower amounts of antigen and a reduced dosing frequency after addition of an adjuvant[83].

Of all currently available adjuvants, aluminium salts (alum) have the longest history in practical vaccination. Alum-based vaccines have a good safety record and are capable of inducing early, high-titer, long-lasting protective immunity. At present, alum is still the most widely used adjuvant in both veterinary and human vaccines. The mechanism of action has been proposed to depend on a depot effect, enabling physical adsorption of antigen onto the alum depots. Furthermore, alum is reported to have direct immunostimulating effects[84]. The relevance of mast cells in alum-mediated adjuvanticity has

been explored[84]. Interestingly, mast cells are found to respond to alum stimulation by releasing histamine and a panel of cytokines including IL-5 and IL-1β. Although by using the mast cell-deficient *Kit*$^{W/W-v}$ mice it is demonstrated that mast cells are not required for the priming of endogenous CD4 and CD8 T cells[84], this does not formally exclude the contribution of mast cells to the adjuvant activity of alum in the wild-type mice as redundant pathways may exist.

However, alum does not seem to be effective for mucosal immunisation, a route that has appreciable advantages compared with routes that require needle injections, i.e. intramuscular or subcutaneous delivery of vaccines. Needle-free mucosal vaccination can be achieved *via* oral, intranasal, sublingual, or intravaginal routes[85-86]. The obvious benefits of mucosal immunisation include avoidance of blood-borne contamination through re-use of syringes and needles as well as the fact that no trained professional personnel are required for vaccine delivery. Furthermore, mucosal immunisation can generate both systemic and mucosal immune responses[85-86]. Strikingly, mucosal immunisation can generate effective secretory IgA even at mucosal sites distant from where the vaccine is delivered[87-88]. For example, nasal immunisation can generate protective mucosal antibodies in the genital tract mucosa, which signifies the advantage of nasal vaccination. As most pathogens enter the body through mucosal surfaces, local mucosal immune responses are critically important in defense against invading pathogens. Therefore, how to achieve strong local protection has become one of the major goals of vaccine development.

As the mucosal route of vaccination, as opposed to the parenteral route, often results in immune tolerance development, potent adjuvants are much warranted. Therefore, the selection of a strong mucosal adjuvant for effective vaccination is vital and possibly as important as the vaccine antigens themselves[85]. A number of strategies are proposed to design mucosal adjuvants. TLR agonists have been tested and these include TLR4 ligand monophosphoryl lipid A[89-90], TLR9 ligand CpG oligodeoxynucleotides (ODN)[91] and the TLR5 ligand flagellin[92]. Bacterial enterotoxins which include cholera toxin (CT) and *Escherichia coli* heat-labile toxin (LT) constitute another major group of experimental mucosal adjuvants[93]. Both CT and LT are composed of five B-subunits (CTB and LTB) and a single copy of the A subunit (CTA or LTA)[94]. The CTA subunit is produced as a single polypeptide chain that is post-translationally modified through the action of a *Vibrio cholerae* protease to form two chains, CTA1 and CTA2, which remain linked by a disulphide bond. CTA1 is enzymatically active by ADP-ribosylating the cell membrane bound Gsα-protein, whereas CTB binds to GM1-gangliosides present on virtually all nucleated cells[95]. CTA2 is responsible for linking CTA into the CTB pentamer[96].

DC are believed to play a central role in the presentation of antigens to naïve T cells, which is a critical process for the development of adaptive immunity following natural infection[97]. As adjuvants are expected to mediate the same consequences as natural infections, quite a number of adjuvant studies are focused on the interaction of adjuvant with DC. Other types of cells have also been described to contribute to adjuvanticity. For example, B cells[98-99], macrophages[100], NK cells and NKT cells[101-103] have also been implicated as targets for vaccine adjuvants.

Given the accumulating evidence suggesting a functional interplay between mast cells and other immune cells such as DC, T cells and B cells in adaptive immune responses, also mast cells have been implicated in adjuvant functions. Indeed, mast cell activators such as c48/80 have been reported as exerting a mucosal adjuvant function[104]. More specifically, c48/80 is demonstrated to be an efficient adjuvant by mobilizing DC to the draining lymph nodes through production of TNF. Successful vaccinations of several animal infection models using c48/80 as adjuvant have now been reported[105-109]. Retention of c48/80 and antigen on mucosal surfaces by chitosan-based nanoparticles can further promote mucosal immunisation[110]. The IL-1 family cytokines such as IL-1, IL-18 and IL-33 have been shown to exert adjuvant function capable of augmenting protection against influenza virus infection[111]. Interestingly, the effect of IL-18 and IL-33 is suggested to be mast cell-dependent[111], which is not surprising as both cytokines can activate mast cells resulting in proinflammatory cytokine production. IL-18 together with IL-2 is potent in expanding the mucosal mast cell pool and the production of mMCP-1, which is critical for parasite expulsion[112]. IL-33 is described as a danger signal that can alert mast cells[113] and keratinocyte-derived IL-33 can stimulate mast cells to produce TNF and IL-6, cytokines critical for defence against herpes simplex virus infection[114]. Polymyxins which are clinically approved antibiotics can activate mast cells and boost immunisation[115]. In a QuilA-adjuvanted cattle vaccination model for protection against nematode infection, mast cells are most likely to be involved in the mechanism of adjuvanticity through the production of granzyme B and granulysin[116]. Synthetic particles harboring TNF, mimicking mast cell granules, have been reported to be powerful adjuvant in a mouse model of influenza[117]. Furthermore, it is suggested that the gold standard mucosal adjuvant CT may stimulate the

release of IL-6 from mast cells boosting humoral immune responses[118].

Although the bacterial enterotoxins have been demonstrated to be powerful mucosal adjuvants experimentally, these substances are precluded from clinical use because of their toxicity and, hence, they have very limited applicability in human vaccines[119-120]. Extensive studies have, however, focused on the detoxification of these molecules using various approaches. For example, site-directed mutagenesis has generated detoxified mutants, such as CT112K, LTG192, LTR72, or LTK63, with little or no enzymatic activity, but with retained adjuvant function in experimental models[121-124]. However, a drastically different approach was applied by Lycke and co-workers who developed an adjuvant based on the intact CTA1 molecule without the B-subunit. The CTA1 is linked genetically to a dimer of the D-fragment of *Staphylococcus aureus* protein A forming the CTA1-DD adjuvant. Thus, CTA1-DD has retained the adjuvant function while the molecule cannot bind to GM1-ganglioside, rendering the molecule nontoxic[125]. In contrast to CT, intranasal administration of CTA1-DD results in neither inflammation nor accumulation in nervous tissues as is found with CT or LT[126]. The adjuvanticity of CTA1-DD has been well documented in various infectious disease models, which include *Chlamydia trachomatis*, influenza, HIV, *Mycobacterium tuberculosis,* and *Helicobacter pylori*[127-133]. The ADP-ribosyltransferase activity is central to the adjuvant effect[134]. In addition, mechanistic studies have identified several mechanisms of action that may explain the adjuvanticity of CTA1-DD *in vivo*. As the DD domain binds to all immunoglobulins, CTA1-DD can target B cells through the B cell receptor, i.e. surface bound immunoglobulins, and promote B cell activation and germinal center development[135]. Moreover, the adjuvant also enhances T cell-independent immune responses[135]. Importantly, CTA1-DD stimulates germinal center formation effectively generating long-lived plasma cells and long-lived B memory cells[136]. Furthermore, also follicular DC and complement activation have been found to be essential elements for the function of this adjuvant[137].

In contrast to intact *Staphylococcus aureus* protein A, which can activate mast cells[40,42], CTA1-DD fails to activate mast cells[138]. However, as the double D domains derived from protein A have binding sites for immunoglobulins, CTA1-DD can bind to all immunoglobulins including IgG[139-140]. We demonstrated that CTA1-DD and IgG may form complexes that are able to activate mast cells through Fcγ receptors, resulting in degranulation and the production of TNF and IL-6. Intranasal immunisation in the presence of CTA1-DD and IgG as an adjuvant can enhance antigen-specific immune responses compared with CTA1-DD alone. Importantly, this enhancement is dependent on mast cells[138]. Furthermore, we demonstrated that only CTMC, but not MMC, can be activated by immune complexes composed of CTA1-DD and IgG. This effect is mediated by FcγRIIIA, an activating receptor that is confirmed to be only expressed on CTMC. Indeed, CTMC are found in the nasal submucosa and these cells are demonstrated to express FcγRIIIA[13].

As MMC are not activated in response to stimulation by IgG immune complexes because of the lack of FcγRIIIA[13], it was intriguing to investigate whether or not MMC could contribute to adaptive immune responses somehow, perhaps using another mechanism. We have recently reported that IgG immune complex-primed MMC can mediate enhanced antigen-specific activation of T cells, possibly providing a cross-presentation mechanism to boost mucosal vaccination[141]. In practical immunisation, this may happen when IgG immune complex-containing vaccine formulations are used.

The development of adjuvants that enhance the potency of subunit vaccines formulated for administration through the mucosal routes is much desired. Dissecting and revealing the molecular mechanisms, through which mast cells precisely control adaptive immune responses to combat microbial infections, may have implications for rationally designing mucosal vaccine formulations. We propose that IgG immune complex-induced mast cell activation may be considered

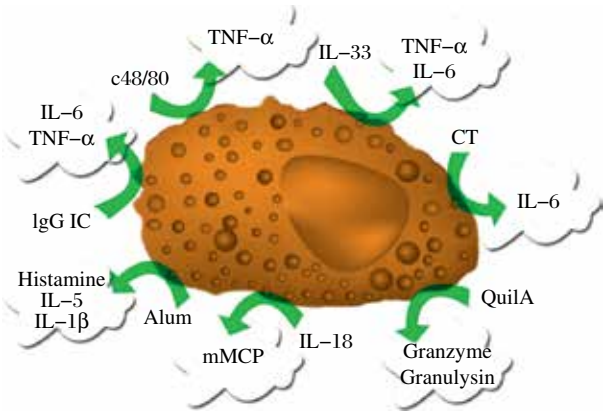

***Fig. 1* Mast cell activation strategies that may be exploited to provide adjuvant activity.** Mast cells can be activated by various factors including compound 48/80 (c48/80), IL-33, IL-18, cholera toxin (CT), QuilA, alum and IgG immune complexes (IgG IC). Upon activation, mast cells release TNF-α, IL-6, IL-5, IL-1β, histamine, mouse mast cell protease (mMCP), granzyme and granulysin that are critical in mobilizing immune responses. However, these are just examples of functional consequences of mast cell activation specific to the findings of those studies addressing roles of mast cells in vaccination cited in this review; the actual spectrum of mediators released by mast cells can be much more dynamic and complex.

as one of the components for mucosal vaccine adjuvants. ***Fig. 1*** summarizes the current knowledge regarding the strategies for the selection of vaccine formulations that target mast cells for enhancing immune responses.

One of the challenges associated with mast cell-mediated immune enhancement, of course, lies in overcoming the complexity of safety issues for the clinical development of the vaccines. The constant threats posed by infectious diseases over millions of years may have driven evolutionary pressure to keep mast cells, despite their adverse properties, *e.g.* in causing allergy, in humans to exploit these cells' beneficial functions in host defense. Our immune system has evolved mechanisms to balance the positive and negative contributions of mast cells to health. It is worth exploring strategies to make use of the adjuvant properties of mast cells to provide high-quality vaccination while minimizing any health-compromising factors.

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