A study of the contribution of mast cells to vaccination

- Regulatory functions of Fcγ receptors

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Cover illustration: CTA1-DD/IgG immune complex targeting mast cells, by Keyu Xiao

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To my parents

ABSTRACT

This thesis aimed to explore the regulatory roles of mast cells in vaccination. Mast cells have been increasingly recognized as important orchestrators of immune regulation in both health and disease, in addition to their classically defined roles in allergic diseases. One of the recently appreciated beneficial roles of mast cells is their involvement in augmenting the adjuvant effects that are critically important in successful vaccination.

This study has focused on the interaction of mast cells with an adjuvant complex composed of IgG and CTA1-DD, a fusion protein consisting of the Al subunit of cholera toxin (CT) linked to a synthetic dimer of fragment-D of *Staphylococcus aureus* protein A (DD). As the DD domain unspecifically binds immunoglobulins, CTA1-DD and IgG form complexes potentially able to activate mast cells through Fcγ receptors. Indeed, CTA1-DD, in combination with polyclonal IgG, induced mast cell degranulation and the production of TNF-α, a cytokine important for the maturation and migration of antigen presenting cells, resulting in enhanced antigen-specific immune responses following immunization.

Furthermore, only connective tissue mast cells (CTMCs), but not mucosal mast cells (MMCs), were found to be activated by CTA1-DD/IgG complexes. This effect was mediated by FcγRIIIA, an activating receptor that has been described to be only expressed on connective tissue mast cells. Indeed, FcγRIIIA-expressing connective tissue mast cells were found in the nasal submucosa. Responses to immunization facilitated by CTA1-DD/IgG were compromised in FcγRIIIA-deficient mice, and in mice pre-treated with a CTMC inhibitor.

Interestingly, MMCs, which were present in mouse nasal mucosa, were not entirely bystanders in CTA1-DD/IgG-mediated adjuvanticity. We discovered that a balanced expression of FcγRIIB and FcγRIIIA was required for mast cells to resist apoptosis mediated by IgG immune complexes. Therefore, MMCs, which only expressed FcγRIIB, but not FcγRIIIA, underwent apoptosis as a result of treatment by CTA1-DD/IgG. MMCs were capable of phagocytosing ovalbumin (OVA), and engulfment of these MMCs by antigen presenting cells (APCs) could occur if the MMCs were induced to apoptose. Finally, the APCs were able to present OVA peptide to OVA-specific T cells. Thus, MMCs may also contribute to vaccination through cross-presentation.

Safety is always a prioritized concern for developing adjuvants, especially when mast cells are involved. Remarkably, CTA1-DD did not function as a superantigen to activate mast cells which had captured IgE molecules with their FcεRI, indicating that CTA1-DD is safe for use in allergic patients in which mast cell FcεRI is occupied by

antigen-specific IgE molecules. Furthermore, CTA1-DD/IgG immune complexes administered intranasally did not trigger systemic anaphylaxis.

In conclusion, CTA1-DD/IgG may target both CTMCs and MMCs through Fcγ receptors to enhance antigen-specific immune responses, probably through two distinct mechanisms. We propose that IgG immune complex-induced mast cell activation may be considered as a component of rationally designed mucosal adjuvants.

Keywords: mast cell, vaccine, adjuvant, CTA1-DD, Fcγ receptor, immune complex, apoptosis

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LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Fang Y, Larsson L, Mattsson J, Lycke N & Xiang Z. Mast cells contribute to the mucosal adjuvant effect of CTA1-DD after IgG-complex formation. *J. Immunol*. 2010; 185(5):2935-2941.

II. Fang Y*, Zhang T*, Lidell L, Xu X, Lycke N & Xiang Z. The immune complex CTA1-DD/IgG adjuvant specifically targets connective tissue mast cells through FcγRIIIA and augments anti-HPV immunity after nasal immunization. *Mucosal Immunol.* 2013; doi: 10.1038/mi.2013.16.

III. Fang Y*, Larsson L*, Bruhns P & Xiang Z. Apoptosis of mouse mast cells is reciprocally regulated by the IgG receptors FcγRIIB and FcγRIIIA. *Allergy.* 2012; 67(10):1233-1240.

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Abbreviations

Alum	Aluminium salt
APC	Antigen presenting cell
BMMC	Bone marrow-derived cultured mast cell
β-hex	β-hexosaminidase
CT	Cholera toxin
CTA	Cholera toxin subunit A
CTB	Cholera toxin subunit B
CTMC	Connective tissue mast cell
DC	Dendritic cell
ELISA	Enzyme-linked immunosorbent assay
FcεRI	Fc epsilon receptor I (high affinity IgE receptor)
FcγR	Fc gamma receptor (IgG receptor)
FcR	Fc receptor
IFN-γ	Interferon γ
Ig	Immunoglobulin
IL	Interleukin
ITAM	Immunoreceptor tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibition motif
i.v.	Intravenous
LAT	Linker for activation of T cells
LPS	Lipopolysaccharide
MHC	Major histocompatibility complex
MMC	Mucosal mast cell
MMCP	Mouse mast cell protease
NLR	NOD-like receptor
MMCP	Mouse mast cell protease
Nlp3	NACHT, LRR and PYD domains-containing protein 3
NAD	Nicotinamide adenine dinucleotide
OVA	Ovalbumin
PAMP	Pathogen-associated molecular pattern
PCA	Passive cutaneous anaphylaxis
PI	Propidium iodide

PRR	Pattern recognition receptor
PS	Phosphatidylserine
RT-PCR	Reverse transcriptase polymerase chain reaction
SCF	Stem cell factor
SHIP	Src homology 2 (SH2) domain-containing inositol phosphatase
SHP	Src homology 2 (SH2) domain-containing protein tyrosine phosphatase
TGF	Transforming growth factor
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor α
TRAIL	Tumor necrosis factor-related apoptosis inducing ligand
7-AAD	7-Amino-actinomycin D

INTRODUCTION

Vaccine and adjuvant

General introduction

A vaccine is a biological preparation that stimulates immunity to one or several antigens that most often are derived from the pathogen itself. The first documented use of a vaccine was the inoculation of a young boy with cow pox to prevent small pox. This was done by Edward Jenner in 1796, hence the term 'vaccine' which is originally derived from the Latin word "*vacca",* meaning "cow". Since then, vaccines have played a crucial role in protecting us against many infectious diseases [1-2]. The eradication of smallpox and the effective control of polio represent two classical success stories of how vaccines have played a major role for improving global health. Nevertheless, the demand for better and more effective vaccines against many infectious diseases is still apparent with infections such as tuberculosis, HIV or malaria as well as vaccines for emerging infections such as dengue fever. 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.

By mimicking the process of natural induction of immune responses occurring during infections, vaccination aims at generating immunological memory against the pathogen, so that the immune system can readily protect against infection, even though re-exposure to the pathogen occurs several years after vaccination. In addition, a hallmark of effective vaccination is the generation of long-lived plasma cells, which can produce neutralizing serum antibodies that can prevent an imminent threat of infection. The importance of B cells for vaccine-induced protection is most often complemented by a critical regulatory role of CD4 T cells, specifically as helper T cells are required for B cell expansion and differentiation. In fact, T cells are indispensable for the induction of high-affinity antibodies and for class-switch recombination from immunoglobulin (Ig) M to down-stream antibodies, predominantly IgG antibodies. Hence, memory CD4 T cells also contribute to the build-up of long term immunological memory. Therefore, vaccines against infectious pathogens rely on both effector arms of the immune system, i.e. antibody-dependent humoral immune responses and T cell-mediated immune responses, which include both CD4 T cell subsets as well as cytolytic CD8 T cells [3].

A number of strategies have been developed for production of effective vaccines. Different routes have been tested for vaccine inoculation, e.g. intramuscular or subcutaneous injections with needles, as well as needle-free methods such as mucosal

vaccination via oral, intranasal, sublingual, or intravaginal routes, or as an aerosol [4-5]. The advantages of mucosal immunization are widely appreciated, and the obvious benefits include avoidance of blood-borne contaminations due to re-use of syringes and needles as well as the fact that no trained professional personnel are required for vaccine delivery. Furthermore, mucosal immunizations can generate both systemic and mucosal immune responses [4-5]. Strikingly, mucosal immunization can generate effective secretory IgA (SIgA) even at mucosal sites distant from where the vaccine is delivered. For example, nasal immunization can generate protective mucosal antibodies in the genital tract mucosa, which demonstrates 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.

There are two principle forms of vaccines: those that are live attenuated vaccines and those that are killed whole pathogens or subcomponent vaccines. Recent cutting-edge technology in genome sequencing and computer-aided prediction of B and T cell recognized epitopes has enabled identification of putative protective vaccine antigens [6-7]. Furthermore, structure-based antigen design has facilitated the rational development of candidate vaccines. Today this is a driving force for vaccine innovation [7]. An advantage of live attenuated vaccines is that they usually stimulate long-term immune responses similar to the natural infection. However, at least in the past there was always a risk of reversion into more virulent organisms, which 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 when 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.

To make killed vaccines effective we need adjuvants which are substances that enhance immune responses and stimulate long-lasting, robust protective immunity. Adjuvant, originally from the Latin word "*adjuvare*", which means "to help", is a substance that can enhance the immunogenicity of an antigen [8]. An adjuvant that is included in the vaccine contributes greatly to the efficacy of the vaccination. An adjuvant affects the immune responses both quantitatively and qualitatively. Importantly, protective immunity following vaccination may be generated with lower amounts of antigen and a reduced frequency of doses after addition of an adjuvant. Furthermore, from a qualitative aspect cross protection to related, heterologous pathogens, can be achieved through epitope spreading provided a strong adjuvant is used. These are examples of critical adaptations of the immune response that can be achieved with appropriately selected adjuvant formulations [9].

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-titre, 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 can have direct immunostimulating effects [10]. Recently, the adjuvanticity of alum was demonstrated to be critically dependent on the inflammasome Nalp3, because the effect of alum was abrogated after eliminating Nalp3-signaling [11-12].

Pattern recognition receptors (PRRs) can detect pathogen-associated molecular patterns (PAMPs). The PRRs are highly conserved microbial structures that frequently have been used when designing vaccine adjuvants [13]. Members of this family include Toll-like receptors (TLRs) that are either expressed on the cell membrane recognizing PAMPs or intracellularly where they recognize translocated intracellular microbial PAMPs such as RNA and DNA. Other family members include NOD-like receptors (NLRs) in cytoplasm that sense nucleotides and RIG-1-like receptors (RLRs) for the detection of dsDNA. Following interactions with their cognate ligands (vaccine adjuvant mimics), TLRs and NLRs initiate a series of signaling cascades leading to cell activation and the build-up of an immune response. One can say that adjuvants are used to mediate the same consequences as natural infection.

Dendritic cells (DCs) are believed to play a central role in the presentation of antigens to naïve T cells. This is termed priming of a T cell response and it is critical for the development of adaptive immunity following both natural infection as well as after vaccination [14]. Therefore, quite a number of adjuvant studies are focused on the interaction of adjuvant with DCs. Other types of cells have also been described to contribute to adjuvanticity. For example, B cells [15-16], macrophages [17], natural killer (NK) cells [18-20] and, more recently, mast cells [21] have also been implicated as targets for vaccine adjuvants.

Similar to natural infection an effective induction of an immune response following vaccination most often requires activation of both innate and adaptive immunity. Therefore, a good adjuvant is expected to be able to stimulate not only innate, but also adaptive immunity. Activation of CD4 helper T cells requires at least two signals from the antigen presenting cell (APC), i.e. signal-1 (antigen presentation) and signal-2 (co-stimulation). Adjuvant may enhance signal-1 by providing a “depot” effect for the antigen so that the availability of antigen may be improved. Adjuvant can also contribute to signal-2 by providing the correct co-stimulation signals [22].

Very few licensed adjuvants are commercially available [4]. Adjuvants facilitating mucosal immunizations have attracted great interest recently given the well-documented merits of mucosal vaccination.

Mucosal adjuvant

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 [4]. TLR agonists form a major group of mucosal adjuvants, which include, for example, TLR4 ligand monophosphoryl lipid A [23-24], TLR9 ligand CPG oligodeoxynucleotides (CPG ODN) [25] or the TLR5 ligand flagellin [26]. Bacterial enterotoxins which have immunomodulatory function, such as cholera toxin (CT) and *Escherichia coli* heat-labile toxin (LT), constitute another major family of mucosal adjuvants [27]. CT is produced by *Vibrio cholerae*, a Gram-negative, comma-shaped bacterium which is the causative agent of the watery diarrhea characteristic of cholera infection [28]. Both CT and LT are AB5-complexes, i.e. they are composed of five B-subunits and a single copy of the A subunit (CTA or LTA) [29]. 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 and ADP-ribosylates the cell membrane bound Gsα-protein, whereas CTB binds to GM1gangliosides present on virtually all nucleated cells [30]. CTA2 is responsible for linking CTA into the CTB pentamer [31].

CTA1-DD

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 vaccines [32-33]. 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, LTR192G or LTK63, with little or no enzymatic activity, but with retained adjuvant function in experimental models [34-36]. However, a drastically different approach was applied by Lycke and co-workers who developed an adjuvant based on the intact CTA1 molecule, but with the B-subunit deleted. The CTA1-DD adjuvant was constructed and CTA1 was linked genetically to a dimer of the D-fragment of *Staphylococcus aureus* protein A. This way the molecule had retained the adjuvant function while CTA1-DD could not bind to GM1-ganglioside [37]. Importantly, CTA1-DD can bind to all immunoglobulins [38-39]. In contrast to CT, intranasal administration of CTA1-DD resulted in neither inflammation nor accumulation in nervous tissues as was found with CT, LT or even

LTK63 [40]. The adjuvanticity of CTA1-DD has been well documented in various disease models, which include *Chlamydia trachomatis*, influenza, HIV and *Mycobacterium tuberculosis* [41-44]. The mechanisms that contribute to the adjuvant effect have been carefully investigated in many different models and also safety aspects of the adjuvant have been tested also in non-human primates. The ADP-ribosyltransferase activity is central to the adjuvant effect [45]. In addition, mechanistic studies have identified several mechanism of action that could 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. Moreover, the adjuvant also enhances priming of T cells [46]. Importantly, CTA1-DD stimulates germinal center formation and effectively generates long-lived plasma cells and long-lived B memory cells [47]. Recently, also follicular DCs and complement activation were found to be essential elements for the function of this adjuvant [48]. Importantly, given the complexity of the adjuvants it is possible that CTA1-DD also exerts other immunoenhancing effects. Replacement of a single amino acid at position 7, *arginine* by *lysine,* (CTA1-R7K-DD) abolishes the NAD-binding and enzymatic property and, thus, abolishes the adjuvant effect of CTA1-DD completely [49]. However, interestingly, this mutant CTA1-R7K-DD molecule has been found to specifically stimulate immune tolerance [50]. By inserting high affinity peptides in the fusion protein this molecule can now serve to induce specific tolerance in the immune system. For example, CTA1-R7K-collagen-DD, when administered in a mouse collagen-induced-arthritis model, can promote the generation of interleukin (IL)-10-secreting regulatory T cells that significantly ameliorate arthritis [51].

Mast cells

General 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 the number of mast cells in the tissue and the severity of the allergic symptoms [52]. In allergy, multivalent antigens bind and cross-link IgE molecules bound to the high-affinity IgE-receptor (FcεRI) expressed on mast cells. This results in cell degranulation and the release of proinflammatory mediator molecules. 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; and (3) De novo synthesized cytokines. In allergy, IgE-mediated activation of mast cells leads to the release of inflammatory mediators in the early phase, resulting in greater levels of epithelial

permeability, mucous production, smooth muscle contraction, vasodilitation and neurogenic inflammation. The immediate response is followed by recruitment of a variety of other immune cells exacerbating the allergic pathology. For example, in diseases such as asthma, mast cells contribute to both the initiation of the acute response and the development of the later chronic stages [52].

Mast cells are derived from hemotopoietic progenitors in the bone marrow and migrate via the blood circulation to tissues 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 [53]. The cellular receptor for SCF is the product of the c-*kit* proto-oncogene. However, immature mouse mast cells can be differentiated *in vitro* from bone marrow precursor cells in the presence of IL-3 without SCF [54]. Mast cells are found scattered in skin, around blood vessels, in mucosal membranes, as seen in e.g. 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. Noteworthy, mature mast cells are capable of differentiating both phenotypically and functionally as a consequence of properties in the microenvironment. For example, inflamed lungs are reported to have more tryptase/chymase-producing mast cells compared to non-inflamed lung tissue in which chymase-producing mast cells are dominant [55-56].

Multitalented cells beyond allergy

In addition to IgE- and FcεRI-mediated cell activation, mast cells can also be activated by a variety of other stimulators, such as IgG immune complexes, cytokines, complement components, neuropeptides, chemical agents, physical stimuli etc, as they express a variety of surface receptors such as Fc receptors (FcRs) and TLRs [57]. These observations, together with the description of a wide spectrum of mast cell mediators provide a foundation for proposals that mast cells may be implicated in almost all aspects of immune responses. Therefore, mast cells are believed to be responsible for numerous physiological and pathophysiological responses beyond their classically defined role in allergic pathology which is mediated mainly through the IgE receptor [58-61]. The malignant expansion and overgrowth of mast cells, i.e. mastocytosis can cause leukemia or sarcoma [62-64], and mast cells are also involved in many other types of cancer [65-68]. Mast cells have also been implicated in diseases such as cardiovascular disease [69-72], autoimmune disease [73] including multiple sclerosis [74-75], rheumatoid arthritis [76-77], diabetes and obesity [78-80]. Accumulations of mast cells are often found in the afflicted tissues or their adjacent boundaries, most often aggravating but in some cases ameliorating immunopathology.

Although still controversial, mast cells have been shown to possess immunosuppressive functions in several studies [81]. Such down-regulated immune responses ascribed to mast cells are mostly associated with cytokines released from mast cells, such as IL-10 and transforming growth factor (TGF)-β [82-83], which have been extensively documented as maintaining homeostasis and limiting inflammatory responses through regulating lymphocyte proliferation, differentiation, and survival [84-85]. Also, mast cells have been implicated in tolerance induction in contact hypersensitivity [83], UV radiation-induced skin inflammation [86] and transplantation [87-89]. The mechanisms for their immunoregulating functions have been studied to some extent. It is known that mouse mast cell protease-4 could have a protective role in allergic airway inflammation [90] and sepsis [91]. Mast cell-derived IL-2 could contribute to the maintenance of suppression in a chronic allergic skin inflammation model by increasing the ratio of regulatory T cells to effector T cells [92]. These findings, especially those suggesting the negative regulation of allergic inflammation, have completely changed the old dogmatic view on the mast cells as being notoriously negative for tissue homeostasis.

Mast cell subtypes

Two major subtypes of rodent mast cells have been characterized, i.e. connective tissue mast cells (CTMCs) and mucosal mast cells (MMCs), based on their tissue localization [93-96]. For instance, skin mast cells and mast cells residing in the peritoneal cavity are CTMCs, whereas mast cells located in the respiratory or gastrointestinal tracts are usually characterized as MMCs. In addition to tissue localization, other properties, such as protease profile, membrane receptors, *in vitro* culture profile, etc, also distinguish these two types of mast cells. However, important for my work is also that CTMCs in mice have been found in the submucosa of some tissues e.g. in the stomach [97].

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. Those 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 the mucosal tissues, resembling mouse MMCs, while MC_{TC} are more closely related to mouse CTMCs which reside in such areas as the skin and small intestinal submucosa [98]. 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 [99]. Table 1 lists the comparisons between the mast cell subtypes in rodents and humans for their major biological features.

Table 1. Heterogeneity of mast cells in rodents and humans

Rodent mast cells	**MMC**	**CTMC**
Location	Mucosal tissue	Serosal surfaces, peritoneal cavity
Granule morphology	Variable in shape and size	Uniform in shape and size
Proteoglycan content	Chondroitin sulfate	Heparin
Dominant protease profile	MMCP-1, 2	MMCP-3, 4, 5, 6, 7, carboxypeptidase
Histamine content	Low	High
Staining with toluidine blue/safranin	Purple/negative	Purple/red
T-cell dependence of hyperplasia	Yes	No
Inhibition by cromolyn and chondroitin sulfate	No	Yes
Response to secretagogues (such as c48/80)	No	Yes
Life span	Short	Long
Growth factor	IL-3	Fibroblast-derived factors, such as SCF
Human mast cells	**MC_T**	**MC_{TC}**
Location	Lung, small intestinal mucosa	Skin, small intestinal submucosa
Proteases	Tryptase	Tryptase, Chymase
Cytokine profile	IL-5, IL-6	IL-4

MMCP, mouse mast cell protease; SCF, stem cell factor.

Mast cell Fc receptors

As mentioned above, the major functional immunoglobulin receptor through which mast cells are activated in allergy is FcεRI, which is the high affinity receptor for IgE. FcεRI is composed of the α, β, and γ chains [100]. Although several types of human cells, e.g. monocytes, Langerhans cells and DCs, also express FcεRI, only mast cells and basophils express the β-subunit which confers stability on the receptor and amplifies the signal that is generated by the γ-subunit [101]. Dislodging the binding of IgE to FcεRI with, e.g. anti-IgE [102-104] or molecularly engineered protein inhibitor [105], has been tested as a promising therapeutic strategy for the treatment of allergic diseases such as asthma.

In addition to FcεRI- and IgE-mediated activation, signaling through IgG receptors (FcγRs) (Fig 1) also contributes to many immune regulatory functions of mast cells [106-108]. Actually, the potential for IgG to activate mast cells was suggested in the 1950s, long before IgE was discovered, as the mechanisms accounting for mast cell-mediated passive cutaneous anaphylaxis [106, 109]. Multiple isoforms of FcγRs have been described [110-111]. In terms of affinity, they can be categorized as high-affinity and low-affinity receptors. In terms of signaling direction, they can be grouped into activating and inhibitory receptors. Two high-affinity IgG receptors, FcγRI (CD64) and FcγRIV, and two families of low-affinity IgG receptors, FcγRII (CD32) and FcγRIII (CD16), have been discovered. FcγRII exists in 3 subtypes, i.e. FcγRIIA, FcγRIIB, and FcγRIIC. FcγRIII is comprised of 2 subtypes, i.e. FcγRIIIA and FcγRIIIB [111]. FcγRIIB is the only FcγR that is conserved in both human and mouse mast cells. Classically, only FcγRIIB is described as an inhibitory receptor [112]. However, recent evidence suggests that FcγRIIIA may also exert functional inhibition [113-114].

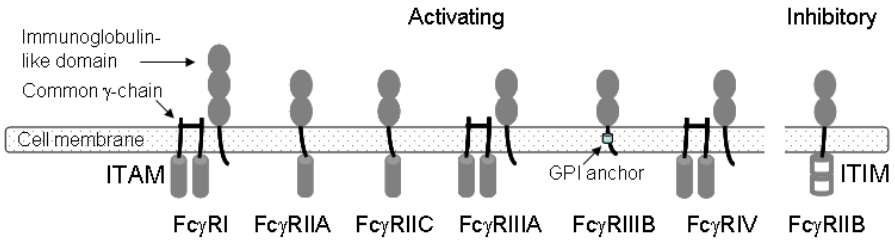

Fig 1. Schematic overview of the structures of Fcγ receptors. Common γ-chains, the signaling adaptor molecules that associate with some of the activating Fcγ receptor complexes for the full function of these receptors. GPI anchor, glycosylphosphatidylinositol, a glycolipid that can be attached to the C-terminus of a protein during posttranslational modification and it helps to anchor the protein to the cell membrane. ITIM, immunoreceptor tyrosine-based inhibition motif. ITAM, immunoreceptor tyrosine-based activation motif.

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Inconsistent expression patterns of FcγRs have been observed among human and mouse mast cells. FcγRI is not constitutively expressed by mast cells, but can be detected on cultured human mast cells upon interferon (IFN)-γ stimulation [115-116] or on mast cells from skin lesions in patients with psoriasis where IFN-γ is enriched [117]. FcγRIIA and FcγRIIB expression by human mast cells is subject to tissue location [106]. Human skin-derived mast cells express FcγRIIA but not FcγRIIB [118]. Human mast cells derived from umbilical cord blood express FcγRIIB [119-120]. In mice, heterogeneity of FcγR expression on mast cells is also observed. FcγRIIB is constitutively expressed on mouse mast cells [121]. IL-3-dependent mouse bone marrow-derived cultured mast cells (BMMCs) do not express FcγRIIIA unless SCF is supplied [122]. Furthermore, it is discovered that mouse CTMCs express both FcγRIIB and FcγRIIIA [122]. In contrast, mouse MMCs only express FcγRIIB [122].

Based on the genomic localization and sequence similarity in the extracellular portion, mouse FcγRIIIA is most closely related to human FcγRIIA [110]. Figure 2 summarizes the expression patterns of FcγRs by mast cells.

	FcγRI	FcγRIIA	FcγRIIB	FcγRIIC	FcγRIIIA	FcγRIIIB	FcγRIV
Human	+[a]	+	+[b]	-	-	-	x
Mouse	-	x	+	x	+[c]	x	-

+, present on mast cells; -, not found on mast cells; x, not expressed in this species.
[a]IFN-γ primed cells; [b]In vitro cultured cells; [c]Connective tissue type mast cells.

Fig 2. FcγR expression on mast cells. Of all the FcγR categories, only FcγRI, FcγRIIA, FcγRIIB and FcγRIIIA have been reported to be expressed by either human or mouse mast cells.

Molecular details associated with the functions of the FcγRs have been revealed [110] (Fig 1). For proper expression and signaling, both FcγRI and FcγRIIIA require one IgG-binding α-chain and two common FcRγ-chains that form a signal-transducing adaptor unit. In contrast, FcγRIIA and FcγRIIB are capable of full signaling with a single α-chain. The α-chain has three (FcγRI) or two (FcγRIIA, FcγRIIB, FcγRIIIA and FcγRIV) immunoglobulin-like extracellular domains that bind the Fc portion of IgG. In addition to the extracellular domains and a transmembrane domain that are shared by all FcγR α-chains, the α-chain of FcγRIIA and FcγRIIB has an intracytoplasmic signaling domain that either contains an immunoreceptor tyrosine-based activation motif (ITAM) or an immunoreceptor tyrosine-based inhibition motif (ITIM). These motifs are important for transducing activating signals or inhibitory signals. The ITIM contained in the cytoplasmic region of FcγRIIB α-chain is responsible for generating inhibitory signals through the recruitment of Src homology 2 (SH2) domain-containing inositol phosphatase (SHIP). All the other FcγRs on mast cells (FcγRI, FcγRIIA and FcγRIIIA) initiate activating signals through ITAM located in the FcRγ-chains (FcγRI and FcγRIIIA) or the intracellular domain of the α-chain (FcγRIIA). In addition to the common γ-chain, FcγRIIIA also contains a signal amplifying β-subunit, which is shared with FcεRI. In contrast to FcRγ, which is widely expressed in hematopoietic cells, expression of FcRβ chain in FcγRs, similarly as in FcεRI, is restricted to mast cells and basophils. The two extracellular domains of mouse FcγRIIB and FcγRIIIA are 95% identical, and high level of sequence homology is also observed between human FcγRIIB and FcγRIIA [106].

FcγRI, the only known high-affinity receptor in humans, has a more narrow spectrum for binding IgG molecules with high-affinity. For example, it binds IgG2a in mice or

IgG1, IgG3 and IgG4 in humans with a high affinity reaching a Ka of about 10^{-8} M [108]. Notably, FcγRI can also be a low affinity receptor for other IgG subtypes. In contrast, the classical low affinity receptors (Ka: 10^{-6} to 10^{-5} M) including FcγRIIA, FcγRIIIA and FcγRIIB demonstrate a broader IgG subclass specificity [108]. In mice, FcγRIIB and FcγRIIIA can bind all IgG subclasses except IgG3. In contrast, FcγRIIA can bind all the human IgG subtypes [108]. FcγRIV is a high-affinity receptor for mouse IgG2a and IgG2b, as it binds monomeric IgG molecules of both classes [111]. So far, no evidence has been obtained indicating the expression of FcγRIV on either human or rodent mast cells.

Cross-linking of activating FcγRs by IgG immune complexes initiates transmembrane signaling by triggering tyrosine phosphorylation of ITAM followed by dynamic protein phosphorylation cascades including the SRC family of tyrosine kinase (Lyn), raft-associated transmembrane adapter proteins, linker for activation of T cells (LAT) and non-T cell activation linker (NTAL), and phosphatidylinositol 3-kinase [110]. Mouse peritoneal mast cells are observed to degranulate upon treatment with IgG1 [123]. Cross-linking of FcγRIIIA on CTMCs results in degranulation and generation of various lipid mediators [124]. In contrast, BMMCs cultured in IL-3 resemble MMCs which do not express FcγRIIIA, and they internalize aggregated IgG without degranulation [125]. Upregulation of FcγRI by IFN-γ on human mast cells followed by treatment with IgG immune complexes results in the release of histamine and cytokines [115].

Inhibitory signaling from FcγRIIB depends on protein phosphorylation cascades that involve phosphatases such as SHIP and SH2 domain-containing protein tyrosine phosphatase 1 (SHP1) [112]. When coaggregated with activating receptors, FcγRIIB inhibits the activating signals from the latter. Various studies have demonstrated the inhibition of IgE- or IgG-mediated mast cell activation by FcγRIIB by using FcγRIIB$^{-/-}$ mice or mast cells derived from these mice [126-128]. Fusion proteins efficiently linking inhibitory signaling from FcγRIIB with activating signaling from FcεRI can dampen IgE-mediated mast cell activation [129-131].

Mast cell apoptosis

Apoptosis, the highly regulated programmed cell death, is one of the pivotal mechanisms to maintain tissue homeostasis. In general mast cell apoptosis is poorly described, but certain mechanisms underlying the apoptosis of these cells have been revealed.

Mast cells are unique among hematopoietic cells in that they do not undergo apoptosis after activation [132-133]. Instead, upon allergic activation, mast cells survive and regranulate [134-136]. It has long been observed that mouse CTMCs have a longer life

span than MMCs [94]. The mechanisms responsible for this difference have not been described. However, it is reported that FcεRI-mediated expression of a Bcl-2 family protein A1 in CTMCs but not MMCs may explain the survival advantage of CTMCs following allergic activation [132, 137]. Noteworthy and in contrast, lymphocytes or even most cells belonging to the innate immune system experience activation-induced apoptosis rather than survival [138].

Apoptosis is accompanied by complex cascades of intracellular events leading to a series of characteristic cellular changes, including transfer of phosphatidylserine to the outer leaflet of the plasma membrane, chromatin condensation and DNA degradation, membrane blebbing, and ultimately the formation of apoptotic bodies, which then are subsequently phagocytosed by macrophages [139]. In contrast to necrosis, which does not rely on energy consumption and always causes inflammatory responses, apoptosis is an energy-dependent process which can avoid tissue inflammation, as apoptotic cells do not release their cellular contents into the surrounding tissue. Instead, apoptotic cells are cleared quickly by phagocytes.

Two main apoptotic pathways have been described [140]. The extrinsic or death receptor pathway is triggered by the engagement of the death receptors (such as Fas or tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL)) with their ligands, followed by activation of caspase-8, leading to caspase-3 activation and finally cell death. The intrinsic or mitochondrial pathway involves a diverse array of stimuli that do not rely on commonly recognized death receptors on cell surfaces but require mitochondrial-initiated events. The factors that can trigger the intrinsic apoptotic processes range from physical stimuli to biological agents such as radiation, toxins and hypoxia, which produce intracellular signals that act directly on targets within the cells. The increased permeability of outer mitochondrial membranes results in cytochrome c release leading to the activation of caspase-9 and the downstream effector caspases. Although caspase activation is recognized as a key element in the apoptotic process, caspase-independent apoptosis has also been reported [141-142]. For example, the caspase-independent apoptosis mediated by the translocation of endonuclease G from mitochondria into nuclei has been shown in mouse mast cells deprived of IL-3 [143].

Mast cell apoptosis can be induced upon deprivation of growth factors such as SCF and IL-3 [144-145]. Cytokines such as IL-10, IFN-γ, and TGF-β1 have the potential to regulate mast cell apoptosis probably by targeting SCF or IL-3 [146-149], suggesting the importance of the local microenvironment in controlling tissue mast cell persistence and density via modulating cytokine production. An increased number of mast cells has been observed in inflamed tissues as a consequence of microenvironmental changes [150-152]. A defect in apoptosis has been found to be associated with certain severe consequences, for example, autoimmune diseases and

cancer [153-154]. Targeting mast cell apoptosis provides an effective therapeutic approach for cancers involving mast cells e.g. mastocytosis [155].

Substances used for modulating mast cell function

Substances that can modulate the function of mast cells are broadly categorized into two groups, i.e. mast cell activators and mast cell stabilizers. In addition to activation by the more classically defined immunological pathways such as Fc receptors and immunoglobulins, a wide variety of substances can also activate mast cells through immunological or non-immunological pathways. These substances include products of complement activation, ligands of TLRs, microbes and their products, neuropeptides (e.g. nerve growth factor and substance P), cytokines, venoms or venom components, analgesics (e.g. codeine), hormones, and small-molecule polymers (e.g. c48/80) [21, 59, 61, 156-157]. In contrast, mast cell activation can be inhibited by the mast cell stabilizers. One of the most implicated stabilizers is cromolyn or disodium cromoglycate that can prevent mast cell degranulation [158]. Recently, the efficacy of cromolyn in stabilizing mouse mast cells has been challenged although it is confirmed to be effective in stabilizing rat mast cells [159]. Flavonoids such as quercetin also has been reported to be able to block mast cell activation [160-161]. Furthermore, MMC granule components such as chondroitin sulfate and heparin can selectively inhibit degranulation of CTMCs [162]. Ketotifen, a noncompetitive H_1-antihistamine, is also described as a CTMC inhibitor [163-164].

The roles of mast cells in host defense

Mast cells are reported to participate in antigen cross-presentation in addition to directly presenting antigens to T cells. Cross-presentation originally refers to a phenomenon, most typically following intracellular microbial infection, when professional APCs ingest infected cells and display the antigens of the microbes present in the infected cells for recognition by T lymphocytes [165]. This is a mechanism that is efficient for presenting the antigens of those microbes that have infected host cells that may not produce all the signals, e.g. major histocompatibility complex (MHC) class II recognition and costimulation, that are needed to initiate T cell activation as professional APCs do. The professional APCs that ingest 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, e.g. surface presence of viral antigens, apoptosis, tumorigenesis etc, will facilitate the ingestion by APCs. In principle, any type of cells that have internalized antigens can participate in cross-presentation upon ingestion by APCs. Importantly, mast cells have been implicated in the phagocytosis of various types of antigens [166-169]. Indeed, one study showed that mast cells can serve as an antigen-reservoir [170]. Involvement of mast cells in antigen cross-presentation has also been reported [170]. *In vitro* cultured BMMCs can internalize FcεRI-IgE bound chicken ovalbumin (OVA)

protein, followed by being phagocytosed by DCs which process and present the OVA peptide to T cells with specific receptors [170]. Induction of BMMC apoptosis is documented to be critical for the efficiency of the phagocytosis [170].

Mast cells constitutively express MHC class I and upregulate expression of MHC class II following stimulation by inflammatory factors such as IFN-γ, TNF-α and lipopolysaccaride [171-172]. Furthermore, mast cells can also express co-stimulators such as CD28, CD80, and CD40 ligand upon activation [173]. Therefore, in addition to participating in cross-presentation, mast cells have the potential to directly activate T cells initiating adaptive immune responses [172-175]. Mast cells have also been demonstrated to present antigen to and activate $CD8^+$ T cells through their MHC class I molecules [176]. The fact that mast cells are capable of participating in both innate and adaptive immunity, and that they are enriched at the mucosal or skin barriers between the body and the external environment, mast cells, similar to skin Langerhans cells, tissue-resident DCs and epithelial cells, are sentinel cells that are probably the first responders to a threat within seconds. The classical examples have been the defense against parasites [177-178]. Furthermore, mast cells have been shown to functionally interact with professional APCs such as DCs and regulate their function mainly through mast cell-derived granular products. For example, histamine is capable of regulating the chemotaxis of immature DCs [179-180] and cross-presentation of extracellular antigens [181]. TNF-α produced from mast cells is critical for DC migration [182-186]. TLR7 ligand-mediated mast cell activation is effective for the migration and maturation of Langerhans cells [187]. Maturation and activation of immature DCs by mast cell-DC direct contact results in the activation of T cells which release IFN-γ and IL-17 promoting Th1 and Th17 responses, respectively [188]. Synthetic particles harboring TNF-α, mimicking mast cell granules, have been reported to be powerful adjuvant in a mouse model of influenza [189].

Mast cells can be activated through direct recognition of microorganisms with the equipped TLRs resulting in distinct outcomes [190-191], by surface proteins such as CD48 [192], by biological substances such as the venom of wasps and snakes [193], or by endogenous inflammatory factors such as cytokines and complement components [194]. 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, differing greatly depending on the stimulus encountered. These mast cell-associated products such as TNF-α, IL-4 and IL-6 are important for the recruitment and stimulation of other participants, e.g. neutrophils, macrophages and T cells, contributing to the clearance of pathogens [195-196]. 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 [197]. TNF-α-independent activation of mast cells by bacterial lipopolysaccharide (LPS) and peptidoglycan has also been reported [198-

199]. 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 [200]. By these diversified mechanisms mast cells exert a pivotal role in the surveillance and elimination of pathogens. 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 [201]. Interestingly, the effect of IL-18 and IL-33 seem to be mast cell-dependent. Given the accumulating evidence that there is a functional interplay between mast cells and other immune cells such as DCs, T cells and B cells in immune responses, also mast cells have been implicated in adjuvant functions. More recently, mast cell activators such as c48/80 have been reported to exert a mucosal adjuvant function [21]. More specifically, c48/80 was demonstrated to be an efficient adjuvant through mobilizing DCs to draining lymph nodes through production of TNF-α. Successful vaccinations of several animal infection models using c48/80 as adjuvant have now been reported [202-204]. Furthermore, also IL-1 family cytokines produced by mast cells have been demonstrated to function as mucosal adjuvants [201].

AIMS OF THE STUDY

The overall objective of this thesis work was to develop new strategies for rationally designing mucosal vaccine adjuvants by exploiting mast cell activation as a mechanism to augment the adjuvant effects.

The specific aims were:

- To investigate whether existing and commonly used adjuvants could activate mast cells.
- To design new adjuvant formulations that could possibly activate mast cells and specifically to test the effect of incorporation of IgG immune complexes into the adjuvant formulation on immune responses.
- To investigate the mechanisms by which mast cells could exert their immune-enhancing effects in response to the new adjuvant formulation, and to determine the mast cell subtypes and surface receptors involved.
- To evaluate the safety profiles of the adjuvant that focused on mast cell activation as part of its mode of action, especially from the perspective of anaphylaxis.

MAIN METHODOLOGY

Mice

Mice used in this thesis included common wild type (WT) control strains, i.e. C57BL/6 and BALB/c, and FcγR knock-out strains, i.e. FcγRIIB$^{-/-}$ mice [127] and FcγRIIIA$^{-/-}$ mice [205], as well as a mast cell-deficient strain that will be described in detail here. C57BL/6-*Kit*$^{w\text{-}sh}$/*Kit*$^{w\text{-}sh}$ (*Kit*$^{w\text{-}sh}$/*Kit*$^{w\text{-}sh}$) mice [206-207].

Mice that specifically lack mast cells have not been created. However, mast cell-deficient strains have been created as a result of natural mutation of c-*kit,* the receptor for SCF that is critically required for the development and survival of mast cells [54]. Two of the c-*kit* mutation-dependent strains have been most widely used: WBB6F1-*Kit*^{W}/*Kit*$^{W\text{-}v}$ (*Kit*^{W}/*Kit*$^{W\text{-}v}$), which have an altered c-*kit* coding region [208-209], and C57BL/6-*Kit*$^{W\text{-}sh}$/*Kit*$^{W\text{-}sh}$ (*Kit*$^{W\text{-}sh}$/*Kit*$^{W\text{-}sh}$), which have an inversion in the regulatory elements upstream of c-*kit* transcriptional site [210-212]. Even though adult *Kit*^{W}/*Kit*$^{W\text{-}v}$ mice and *Kit*$^{W\text{-}sh}$/*Kit*$^{W\text{-}sh}$ mice are profoundly deficient in mast cells, these mice also demonstrate other abnormalities owing to the lack of normal signaling from the Kit receptor. In contrast to *Kit*^{W}/*Kit*$^{W\text{-}v}$ mice [94, 208, 213], the phenotypic abnormalities in *Kit*$^{W\text{-}sh}$/*Kit*$^{W\text{-}sh}$ mice are generally milder: they are neither anaemic nor sterile, but exhibit neuthophilia and splenomegaly [211, 214-215]. So far, the sash mice present a relatively favorite animal model for the *in vivo* investigation of mast cell biology [216]. Therefore, we chose to use the *Kit*$^{W\text{-}sh}$/*Kit*$^{W\text{-}sh}$ model in our study. Homozygotes of *Kit*$^{W\text{-}sh}$/*Kit*$^{W\text{-}sh}$ mice are white with black eyes and some pigment around the ears, while heterozygotes are black with a white sash at the midline, and therefore they have acquired the name 'sash' mice [212]. As the c-*kit* gene is still expressed in the embryos of *Kit*$^{w\text{-}sh}$/*Kit*$^{w\text{-}sh}$ mice resulting in an age-dependent abolishment of c-*kit* gen expression in these mice, an inverse relationship between the tissue mast cell number and the age of the mice is observed [207]. Therefore, importantly, *Kit*$^{w\text{-}sh}$/*Kit*$^{w\text{-}sh}$ mice older than 8 weeks were used.

As mentioned above, the c-*kit* loss-of-function mutation-associated mast cell deficiency affects cell lineages other than mast cells. Therefore, defects in these mice may not necessarily be due to the lack of mast cells. Therefore, confirmation of the role of mast cells is often achieved through the use of the so-called "mast cell knock-in" model, i.e. the selective reconstitution of mast cell deficiency in these mice by engraftment of genetically compatible, *in vitro*-derived mast cells through the intravenous, intraperitoneal, or intradermal routes without repairing the other defects. Restoration of the mast cell population has been achieved in the skin, peritoneum, stomach and lung [208-209, 211, 217-218]. However, a major issue in such knock-in models is whether this technique can reconstitute mast cells qualitatively as well as

quantitatively in the target organs. For example, reconstitution failed to restore mast cells in several organs, e.g. the tongue and trachea of the sash mice [217], and the brain, spinal cord, lymph nodes, heart, and nasal mucosa in the $Kit^{W}/Kit^{W\text{-}v}$ mice [218]. Despite these limitations, the *in vivo* roles of mast cells in certain pathophysiological models have been established by studying these genetically mast cell-deficient mouse models. Further confirmation of the roles of mast cells depend on the next generation of mast cell-deficient mouse models which are in development [219].

Mast cell activation

A variety of factors can target mast cells and induce activation of these cells with defined outcomes. Generally, mast cell activation can be detected by measuring cell degranulation or cytokine production. One of the most convenient readouts for mast cell degranulation is the release of β-hexosaminidase (β-hex) [220], which was determined by a spectrophotometer-based colouremetric assay measuring the conversion of the enzyme's substrate, p-nitrophenyl N-acetyl-b-D-glucosaminide [221]. As for the cytokine production, TNF-α was chosen as the most important cytokine for the adjuvant effect. TNF-α released from mast cells can be routinely detected by enzyme-linked immunosorbent assay (ELISA). However, the fact that CTA1-DD binds to all immunoglobulins resulting in high background staining precludes ELISA for measuring TNF-α in this project. Instead, TNF-α production was detected with intracellular flow cytometry analysis. TNF-α mRNA was measured by real-time reverse transcriptase polymerase chain reaction (RT-PCR). In some experiments, IL-6 production by activated mast cells was also measured.

Mast cell apoptosis

Mast cell apoptosis can be detected by various approaches based on the physiological process of apoptosis [222], such as Annexin V binding and propidium iodide incorporation which were used in this thesis. Annexins are a family of calcium-dependent phospholipid-binding proteins that preferentially bind phosphatidylserine (PS). In healthy cells during steady state, PS is asymmetrically distributed in the inner leaflet of the plasma membrane. However, PS is translocated to the extracellular membrane leaflet during apoptosis which provides targets for phagocytosis. When exposed on the outer surface of the membrane, PS can be readily bound by Annexin V in a calcium-dependent manner, which is indicative of apoptosis. Briefly, for Annexin V binding assay, following surface marker (Kit and FcεRI) staining, mast cells were stained by fluorescence-conjugated Annexin V in a Ca^{2+} enriched solution. 7-Amino-actinomycin D (7-AAD) was always included in the Annexin V staining to detect dead cells. Of note, live, degranulating mast cells also expose PS to the outer leaflet of the cell membrane [223]. Caution has been raised regarding the measure of mast cell apoptosis following activation. However, mast cell degranulation is a relatively rapid

process and cells start to regranulate several hours later [224]. The time point in our study for observing mast cell apoptosis was a 16-hour overnight incubation following IgG immune complex treatment. Therefore, PS exposure should represent cell apoptosis but not degranulation. In some experiments, cells were fixed and permeabilized followed by incubation with propidium iodide (PI) to measure the PI intercalation, also indicative of cell apoptosis.

Passive cutaneous anaphylaxisis

Passive cutaneous anaphylaxis (PCA) refers to an artificial anaphylactic reaction in experimental animals, most often rodents. It is a mast cell-dependent model which is nowadays most often used to demonstrate the immediate dermal response to an allergen-IgE interaction. After sensitization with IgE, the animals are injected intra venously (i.v.) with the Evans blue dye together with the antigen that the IgE recognizes. Reaction of the antigen with skin mast cell-fixed IgE causes the release of vasoactive granular contents, e.g. histamine, from mast cells, which increases vascular permeability and permits leakage of the antigen-bound dye, resulting in a blue spot at the site of the intradermal injection.

This model can also be modified to demonstrate the activation of mast cells by IgG immune complexes. In fact, mast cell activation by IgG had been tested as an approach to initiate PCA in the 1950s, long before IgE was discovered [106]. In this thesis, the effect of IgG/CTA1-DD complexes on the dye extravasation was tested. The ears of these mice were injected with PBS or IgG on one side and IgG/CTA1-DD on the other, followed by an i.v. injection of Evans blue. Mouse ears were removed 20 min after Evans blue injection and the dye extravasation was quantified as reported [225]. In some experiments, to test if CTA1-DD could replace an antigen that the IgE recognizes, own to its unspecific binding potential for immunoglobulins, in cross-linking the IgE bound to FcεRI, and thus possibly activating mast cells, mice received IgE sensitization on both ears. The next day CTA1-DD was injected intradermally in one ear and PBS in the other. Mice were immediately injected i.v. with Evans blue.

Systemic anaphylactic assay

The most commonly used classical method to investigate systemic anaphylaxis is to monitor the consequence of IgE- and FcεRI-based mast cell activation in mice. Mast cells respond to FcεRI cross-linking by immediately degranulating their granular contents, resulting in cascades of events such as dilation of blood vessels which culminates in reduced heart rate and blood pressure, paralleled with a significant drop of body temperature that can be measured with a thermometer with a probe for the mouse anus.

In a typical mouse systemic anaphylaxis model, mice were sensitized systemically with an injection of antigen-specific IgE i.v., followed by injection of the antigen i.v. the next day. The rectal temperature was then recorded using a thermometer. In this thesis, to investigate whether the administration of CTA1-DD/IgG as an adjuvant would initiate systemic anaphylaxis, rectal temperatures were measured at various time points after intranasal or intravenous administration of CTA1-DD/IgG. The results were expressed as changes in temperature from the baseline values.

Tissue mast cell identification

Nasal tissue mast cells

As we intended to develop an effective mucosal immunization strategy, e.g. using the intranasal route by exploiting mast cell activation, it is of great interest to investigate mast cells in the mucosal tissues, e.g. nasal tissues. A technical hindrance to the study of nasal tissues by sectioning and microscopy based approaches is that a decalcification step is required to remove the turbinates, which usually involves harsh treatment of the tissues which may damage some protein epitopes. Therefore, we relied on flow cytometry analysis to identify and analyze mast cells in the nasal tissue. The unskinned mouse snout was finely minced and digested with Liberase and DNase I. After passing through a 70-μm filter and lysis of the red blood cells, a single cell suspension was prepared for fluorescence staining and flow cytometry analysis.

Peritoneal mast cells

Mouse peritoneal mast cells resemble mature CTMCs. They can be used directly or after further culture to certain purity in medium enriched with specific growth factors [188]. Thus, in this thesis work, mouse peritoneal lavage fluid was harvested and peritoneal mast cells were either directly used or cultured.

Antigen cross-presentation

The potential of mast cells to phagocytose OVA was determined by confocal microscopy. Mast cell-mediated antigen cross-presentation was carried out by an initial incubation of mast cells with OVA in the presence or absence of anti-OVA IgG for 2 days followed by extensive washing to remove unphagocytosed OVA. Next, mast cells were mixed with splenocytes from OT-II mice that contained T cells with engineered T cell receptor specific for the OVA peptide 323–339. The cell mixture was incubated for another 2 days. The efficiency of antigen cross-presentation was determined by CD69 expression, a marker that specifically measures T cell activation, on the T cells from the cell mixture.

RESULTS

McLachlan et al. demonstrated that classical mast cell activators, such as C48/80, could exert a potent vaccine adjuvant function [21]. The proposed mechanism was associated with production of inflammatory mediators, e.g. TNF-α, which activated adjacent DCs residing in the microenvironment. This resulted in augmented DC migration to the draining lymph nodes, where adaptive immune responses could be initiated. This finding clearly emphasizes the beneficial effects that mast cells may have. Their study prompted us to investigate whether the mechanism can be further used to improve or explain the effects of mucosal adjuvants and the effects of CTA1-DD, in particular. We first tested a group of well known mucosal adjuvants to determine whether they could directly activate mast cells. However, it turned out that none of these adjuvants could activate mast cells, including mouse bone morrow-derived cultured mast cells (BMMCs) or mouse mast cell lines (C57 and MC/9). For these studies we used degranulation or TNF-α production as our readout. By contrast, because CTA1-DD binds to all immunoglobulins including IgG-subclasses, in particular, we next asked whether CTA1-DD or an immune complex with CTA1-DD/IgG could interact with mast cells and thus provide augmented adjuvanticity.

CTA1-DD/IgG activates connective tissue mast cells to produce TNF-α (Paper I)

Given the fact that mast cells harbor FcγRs which can be cross-linked by IgG immune complexes and the fact that the DD domain of CTA1-DD, which is derived from *Staphylococcus aureus* protein A, can bind immunoglobulins, we speculated that CTA1-DD mixed together with IgG could form immune complexes that could activate mast cells. Indeed, CTA1-DD complexed with IgG was able to activate the mouse mast cell line C57 as measured by TNF-α production and β-hexosaminidase release, a measure of mast cell degranulation (Fig 3). Furthermore, mouse tissue mast cells were also demonstrated to respond to stimulation by CTA1-DD/IgG. Using a modified mouse skin PCA model that is dependent on mast cell degranulation, we also confirmed the activation of mouse ear tissue mast cells by CTA1-DD/IgG. Similarly, CTA1-DD/IgG degranulated nasal tissue mast cells, as was demonstrated by microscopic analysis of tissue sections.

FcγRIIIA expressed on CTMC is critical for mast cell activation by CTA1-DD/IgG (Paper II)

Mouse mast cells can be subgrouped into connective tissue mast cells (CTMCs) and mucosal mast cells (MMCs). Importantly, *in vitro* models of these two subtypes can be readily obtained by subculturing mouse bone marrow cells in the presence of specific and distinctive cytokine compositions. Therefore, we next tested the responsiveness of

these two subtypes to the treatment of CTA1-DD/IgG immune complexes. Clearly, it was CTMCs, but not MMCs, that could be activated by these immune complexes. We extensively characterized CTMCs and MMCs in our culture systems. Consistent with literature reports, these two subtypes of mouse mast cells differed in many respects, including the amount and size of granules, surface expression levels of FcεRI and Kit, and extent of degranulation as a result of FcεRI cross-linking. Specifically, the expression levels of the low affinity IgG receptor FcγRIIIA were substantially stronger on CTMCs than on MMCs. Furthermore, the C57 cell line that could be activated by the immune complex also expressed a higher level of FcγRIIIA.

In subsequent studies we wanted to find out which features distinguished CTA1-DD/IgG immune complex interactions with CTMCs from those with MMCs. Based on the differential expression patterns of FcγRIIIA on CTMCs and MMCs, we investigated whether FcγRIIIA was absolutely required for the activation of mast cells by CTA1-DD/IgG immune complexes.

Our data demonstrated that CTA1-DD/IgG failed to bind MMCs, which do not express FcγRIIIA. As a negative control we also analyzed CTMCs from mice that were deficient in FcγRIIIA, or wild-type CTMCs treated with an FcγRIIIA-neutralizing antibody. Taken together, these results indicated that FcγRIIIA was the critical molecule for the interaction of CTMCs with CTA1-DD/IgG. Such interaction induced TNF-α and IL-6 expression by CTMCs (Fig 3).

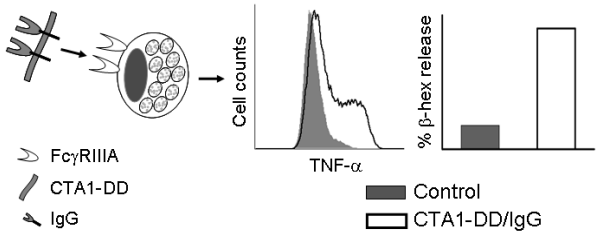

Fig 3. CTA1-DD/IgG immune complexes activate mast cells. Treatment with CTA1-DD/IgG immune complexes resulted in the degranulation (e.g. β-hexosaminidase (hex) release) and cytokine production (e.g. tumor necrosis factor (TNF)-α) from connective tissue mast cells that expressed FcγRIIIA. Shown is an example using the mouse mast cell line C57 which is characteristic of a connective tissue mast cell phenotype.

Identification of CTMC in mouse nasal tissue (Paper II)

We speculated that a prerequisite for CTA1-DD/IgG to augment the adjuvant effect through activation of mast cells was that CTMCs could be found in tissues accessible

through mucosal application. Thus we asked if our adjuvant complex could engage CTMCs after nasal administration. Indeed we found CTMCs in the submucosa of mouse nasal tissue by microscopy and flow cytometry. To confirm FcγRIIIA expression, single cell suspensions obtained from de-skinned mouse nasal tissues were prepared. These studies revealed two distinct mast cell populations: FcγRIIIA-expressing CTMCs and MMCs lacking FcγRIIIA. Mice deficient in mast cells were devoid of both these populations in the nasal mucosa.

Intranasal immunization adjuvanted with CTA1-DD/IgG enhances immune responses (Papers I and II)

We demonstrated that intranasal delivery of antigen together with CTA1-DD/IgG complexes further enhanced serum antigen-specific IgG antibody responses as compared to immunization adjuvanted with CTA1-DD alone. This CTA1-DD/IgG complex-mediated enhancement was mast cell-dependent because it was absent in mast cell-deficient $Kit^{W\text{-}sh/W\text{-}sh}$ mice. Moreover, the augmented adaptive immune response was dependent on CTMCs and FcγRIIIA, as mice deficient in FcγRIIIA, or mice that were treated with chondroitin sulfate, a CTMC stabilizer [162], failed to exhibit an enhanced immune response following immunization adjuvanted with CTA1-DD/IgG complexes (Fig 4).

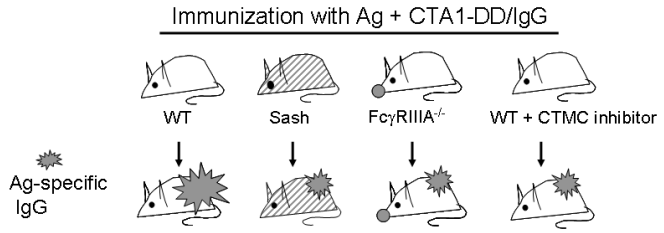

Fig 4. CTA1-DD/IgG augments mast cell-dependent antigen (Ag)-specific immune responses. Mice were immunized with Ag adjuvanted with CTA1-DD/IgG. Ag-specific IgG in serum was measured by ELISA. Compared with the wild-type (WT) mice, compromised serum Ag-specific IgG production was observed in sash mice that lacked mast cells, FcγRIIIA$^{-/-}$ mice or mice pre-treated with chondroitin sulphate (a CTMC inhibitor).

Mucosal mast cells may contribute to the adjuvant effects of CTA1-DD/IgG immune complexes through cross-presentation of antigen (Paper III and unpublished data)

While our studies revealed that only CTMCs that expressed FcγRIIIA could be activated by CTA1-DD/IgG complexes resulting in greater immune responses, we asked whether there was a way by which also MMCs could participate in an

augmented CD4 T cell response following nasal immunization. Mast cells have classically been described as a cell type that can phagocytize [166-169]. We demonstrated that MMCs could efficiently phgocytize OVA (Fig 5). It is likely that mast cells may participate in antigen presentation when mast cells that have phagocytized antigens are engulfed by professional APCs. Interestingly, it has been reported that apoptosis of mast cells increases the efficiency of antigen cross-presentation [170].

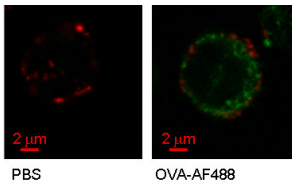

Fig 5. Mucosal mast cells can internalize ovalbumin (OVA). Mucosal mast cells were incubated in the presence or absence of Alexa Fluor 488-conjugated OVA (OVA-AF488, green) overnight. Cell membrane expression of Kit was determined by staining with biotin-conjugated anti-Kit antibody followed by staining with streptavidin-conjugated Alexa Fluor 594 (red) to distinguish mast cells.

Homotypic cross-linking of FcγRIIB, not engaging any activating receptors, induces apoptosis of B-lineage cells [226-228]. It was interesting to investigate the fate of mast cells when FcγRs on these cells were cross-linked. It is easier to study the effect of FcγRs on B-lineage cells as FcγRIIB is the only FcγR on these cells. Indeed, it is more difficult to address the roles of FcγRs on mast cells because they may express more than one type of FcγRs. For example, mouse mast cells may express both FcγRIIB and FcγRIIIA. We discovered that aggregation of one of these two receptors by IgG immune complexes, in the absence of the other, induced apoptosis of mouse mast cells (Fig 6). Interestingly, mast cells with a balanced expression of both FcγRIIB and FcγRIIIA were able to resist apoptosis (Fig 6).

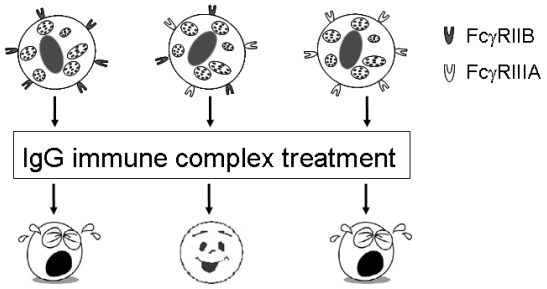

Fig 6. The effect of Fcγ receptor expression profile on IgG immune complex-mediated apoptosis of mast cells. Mast cells expressing both FcγRIIB and FcγRIIIA resisted IgG immune complex-mediated apoptosis (middle panel), whereas those lacking one of the Fcγ receptors were vulnerable to induction of apoptosis (left and right panels).

Consistently, we found that MMCs (with only FcγRIIB) but not CTMCs (with both FcγRIIB and FcγRIIIA) responded to induction of apoptosis by IgG immune-complexes composed of OVA and anti-OVA IgG. Moreover, we demonstrated that CTA1-DD complexed with IgG also induced apoptosis in mouse MMCs (Fig 7).

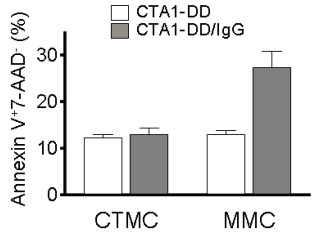

Fig 7. CTA1-DD/IgG induces apoptosis in mucosal mast cells (MMC), but not connective tissue mast cells (CTMC). Cells were treated with CTA1-DD alone or CTA1-DD/IgG and cell apoptosis was revealed by surface staining with Annexin V and 7-AAD by flow cytometry. Apoptotic cells were Annexin V$^+$/7-AAD$^-$.

We, therefore, designed an experiment to analyze whether mast cells could participate in antigen presentation and priming of CD4 T cells. MMCs were incubated with OVA either in the presence or absence of anti-OVA IgG for 2 days followed by extensive washing. Next, OT-II mouse spleen cells were mixed with the MMCs for another 2 days. MMCs treated with OVA and anti-OVA IgG resulted in an increased efficiency of T cell priming as assessed by CD69 expression on the OVA peptide-specific T cells (Fig 8).

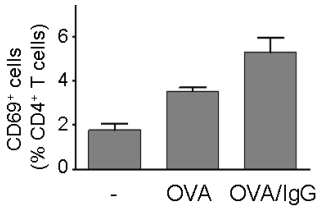

Fig 8. Mucosal mast cells (MMCs) mediate greater efficiency of antigen cross-presentation following incubation with ovalbumin (OVA) complexed with anti-OVA IgG. Splenocytes from OT-II mice were incubated for 48 hours with MMCs that were pre-treated for 48 hours with medium control, OVA alone or OVA together with anti-OVA IgG (OVA/IgG). T cell activation was shown as percentages of CD69$^+$ cells among all CD4$^+$ cells analyzed by flow cytometry.

DISCUSSION

Mast cells have long been recognized for their critical involvement in allergic reactions. However, recent studies have given us reason to think about mast cells also as favorable for a number of host reactions, some of which appear to be crucial for protection against infections. Indeed, humans that lack mast cells have never been reported, arguing that the positive effects of mast cells are required for health and survival. Given the evidence that these cells were found to be involved in phagocytosis and defense against helminth infection decades ago, and the more recent revelation that mast cells contribute to both innate and adaptive immunity by releasing a diverse array of granular contents, it is surprising that not more attention has been given to mast cells in vaccine development earlier. Mast cells are strategically positioned in the interface between the external environment and the host. Indeed, mast cells in skin or mucosal barriers are increasingly being recognized as important contributors to defense mechanisms against infections. Against this background, it is also logical that mast cells could be exploited for augmenting mucosal vaccine efficacy [21]. Mast cells are a heterogeneous type of cells. Although it was suggested back in the 1960s that rodent mast cells could be divided into CTMCs and MMCs based on tissue location and histochemical staining reflecting distinct granular protease contents [95], the functional differences between these two subtypes are less well defined and have only recently been given attention.

This thesis work entertained the hypothesis that some of the mucosal adjuvants already in experimental use actually might exert adjuvanticity by activating mast cells. However, we failed to observe any discernable activation of mast cells by these adjuvants. This could have been due to the fact that we screened for these effects using a mouse mast cell line, which may not necessarily reflect the *in vivo* effects of these adjuvants. In any case all of the effects on mast cells that we have studied have been with CTA1-DD, which is a promising mucosal adjuvant. We demonstrated that when mixed with IgG, CTA1-DD immune complexes activated murine CTMCs through FcγRIIIA, which resulted in TNF-α release. Most importantly, immunization with CTA1-DD/IgG induced mast cell-dependent enhancement of antigen-specific immune responses.

From an adjuvant activity point of view it was clear that only CTMCs carried FcγRIIIA, while MMCs could also be involved in enhancing immune responses by being involved in antigen presentation by DCs after induced apoptosis. Hence, IgG immune complex-mediated mast cell apoptosis, could augment DC antigen-presentation. Furthermore, a balanced expression of FcγRs on mast cells could be important for determining apoptosis or survival. All mouse mast cells express FcγRIIB, and some of them may also express FcγRIIIA. We have speculated that mast

cells with the expression of only one of the receptors may be more susceptible to IgG immune complex-mediated apoptosis as opposed to those bearing both receptors. Indeed, we have documented that due to the lack of FcγRIIIA, MMCs proceed to apoptosis following treatment with IgG immune complexes. This is in contrast to the resistance to apoptosis exhibited by CTMCs, which express both FcγRIIB and FcγRIIIA.

Our *in vitro* data clearly show that CTA1-DD/IgG could not activate MMCs, but instead induced apoptosis of these cells. We showed that MMCs are capable of internalizing OVA, arguing in favor of a role for antigen-presentation provided DCs can engulf apoptotic material from mast cells. OVA pre-treated MMCs contributed to the activation of OVA-specific T cells, and this activation was even more pronounced when MMCs were pre-treated with OVA/anti-OVA immune complexes, which is indicative of the contribution of IgG immune complex-induced mast cell apoptosis in this process. Therefore, it is possible that MMCs expressing FcγRIIB are also involved in the adjuvant effects of CTA1-DD/IgG complex (Fig 9). However, my prediction would be that MMC-mediated cross-presentation may only represent a minor contribution to the adjuvant effect because the CTMC inhibitor chondroitin sulfate substantially inhibited the enhanced adjuvant effect.

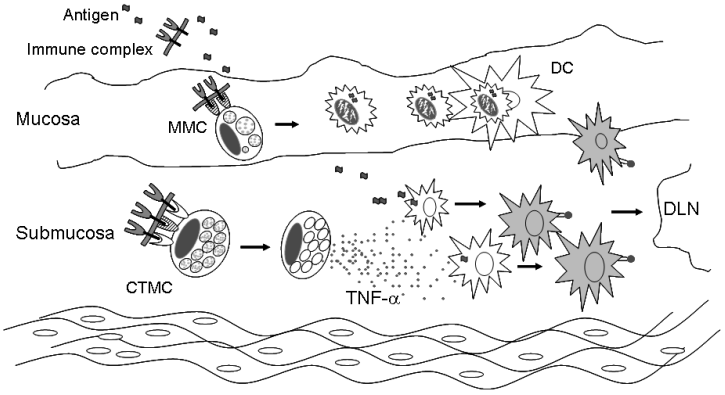

Fig 9. Schematic overview of the proposed mechanisms underlying IgG immune complex- and mast cell-mediated adjuvant activity. Following intranasal immunization, IgG immune complexes may activate submucosa-residing connective tissue mast cells which express both FcγRIIIA and FcγRIIB resulting in the release of cytokines, e.g. tumor necrosis factor-α (TNF-α), that are important for the maturation and migration of dendritic cells (DC). DCs that have engulfed and processed the immunizing antigen next move to the draining lymph nodes (DLN) where presentation of the processed antigen peptide to T cells (not depicted) takes place. Alternatively, mucosal mast cells which are present in the mucosa may phagocytose the immunizing antigen and undergo apoptosis upon treatment with IgG immune complexes due to their expression of only FcγRIIB. Apoptotic mast cells are engulfed by DCs and cross-presentation of the antigen to T cells (not depicted) may occur.

Of note, the presence of FcγRIIB in the context of FcγRIIIA-mediated activation of mast cells might antagonize the activating signaling from FcγRIIIA [112]. This seems to suggest that FcγRIIB is only a negative contributor in the system. However, our finding reveals that this receptor did have a "positive" meaning for the activation in the scenario of immune enhancement mediated through mast cell activation because a sustained cell survival is required for *de novo* synthesis and release of cytokines. In the absence of FcγRIIB, FcγRIIIA-signaling may lead to apoptosis. Indeed, our data demonstrated that IgG immune complexes (e.g. OVA/anti-OVA or CTA1-DD/IgG) were capable of inducing apoptosis of CTMCs derived from mice deficient in FcγRIIB expression. This could, in fact, be explained by the same or a different mechanism of action as that of FcγRIIB-induced apoptosis.

Antibodies can mediate negative or positive feedback regulation of immune responses [229]. Thus, antigen-specific antibodies administered together with the antigen can modulate the immune responses. Most well known is the impact of IgG that can dampen responses to large particulate antigens. By contrast, soluble protein antigens given together with specific antibodies may enhance immune responses and exert an indirect adjuvant function [230]. Although the precise mechanisms of antibody feedback regulation are poorly defined, positive feedback regulation driven by IgG1, IgG2a, and IgG2b involves FcγRs [229, 231]. In contrast, IgG3 and IgM modulation depend on complement activation to enhance antibody responses [229, 232-233]. So far, most of the previous studies addressing antibody feedback regulation have been carried out using the intravenous route for antigen and antibody administration. Our observations with IgG-mediated mast cell- and FcγRIIIA-dependent augmentation clearly provides evidence that immune-complex regulatory feedback also exists at mucosal sites as found after mucosal immunization.

CTA1-DD/IgG either intranasally or intradermally induced mouse tissue mast cell degranulation. Therefore, we performed a series of experiments to assess the safety of CTA1-DD/IgG. We could show that CTA1-DD/IgG was safe when given intranasally or intravenously as no systemic anaphylactic reactions were recorded. Because CTA1-DD can bind virtually all immunoglobulins including IgE [38, 45], this raises a concern that CTA1-DD may interact with IgE molecules, which could bind to FcεRI on mast cells and result in systemic mast cell degranulation and anaphylaxis. However, we did not observe degranulation or TNF-α production from mast cells following treatment with CTA1-DD /IgE complexes. This was in contrast to CTA1-DD/IgG, which activated tissue mast cells. Indeed CTA1-DD/IgE had no activating function on mast cells, which could be due to the fact that CTA1-DD binds IgE with a lower affinity than IgG [38, 45]. An alternative explanation could be that FcεRI and CTA1-DD compete for the same binding epitope on IgE, which would result in poor FcεRI cross-linking.

We have shown that an enhanced adjuvant effect of CTA1-DD/IgG complexes is mediated by mast cells through interaction with the FcγRs. Because there are considerable species differences and discrepancies, and also complexity of mast cell FcγR expression in different cell types exists, caution should be taken when extrapolating mouse data to humans. My thesis has focused on the mouse model. However, it could be entirely possible to exploit mast cells for adjuvanticity in human nasal vaccines. Firstly, mast cells are reported to reside in human nasal mucosa [93]. Secondly, human mast cell subgroups correlate to their rodent counterparts and human tryptase-positive mast cells are referred to as MC_T, which resemble mouse MMCs, whereas those that contain both tryptase and chymase are termed MC_{TC}, which are related to mouse CTMCs [234]. Thirdly, also human mast cells express unique patterns of FcγRs, although they do not express FcγRIIIA, a critical factor in the augmented adjuvant effect by CTA1-DD/IgG in mice. But human mast cells can possibly express both FcγRIIA and FcγRIIB [106]. Based on the genomic localization and sequence similarity in the extracellular portion, human FcγRIIA is most closely related to mouse FcγRIIIA [110]. Therefore, our theory brought forward for a balance between signaling via FcγRs in murine mast cells may also hold true for human mast cells. Furthermore, FcγRI expression in human mast cells can be influenced by IFN-γ, which, in addition, could also contribute to a regulatory mechanism exploited by nasal vaccines [115].

In conclusion, our *in vitro* and *in vivo* data clearly show that CTA1-DD/IgG may target both CTMCs and MMCs through FcγRs. On the one hand, the immune complexes activate CTMCs bearing FcγRIIIA, leading to cytokine release from CTMCs; on the other hand, immune complexes induce apoptosis of MMCs. Whereas the latter may augment DC presentation of antigens associated with apoptotic cells, both types of mast cells may, in fact, contribute to the augmented adjuvant effect of CTA1-DD/IgG. I propose that IgG immune complexes that target mast cells may be considered when designing new mucosal vaccine adjuvant formulations to achieve additive or even synergistic enhancing effects.

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REFERENCES

1. Nabel, G. J. 2013. Designing Tomorrow's Vaccines. *New England Journal of Medicine*. 368:551-560.
2. Levitz, S. M. and D. T. Golenbock. 2012. Beyond empiricism: informing vaccine development through innate immunity research. *Cell*. 148:1284-1292.
3. Igietseme, J. U., F. O. Eko, Q. He, and C. M. Black. 2004. Antibody regulation of Tcell immunity: implications for vaccine strategies against intracellular pathogens. *Expert Rev Vaccines*. 3:23-34.
4. Lycke, N. 2012. Recent progress in mucosal vaccine development: potential and limitations. *Nature reviews. Immunology*. 12:592-605.
5. Holmgren, J. and A.-M. Svennerholm. 2012. Vaccines against mucosal infections. *Current Opinion in Immunology*. 24:343-353.
6. Sette, A. and R. Rappuoli. 2010. Reverse vaccinology: developing vaccines in the era of genomics. *Immunity*. 33:530-541.
7. Dormitzer, P. R., G. Grandi, and R. Rappuoli. 2012. Structural vaccinology starts to deliver. *Nature reviews. Microbiology*. 10:807-813.
8. Stills, H. F., Jr. 2005. Adjuvants and antibody production: dispelling the myths associated with Freund's complete and other adjuvants. *ILAR journal / National Research Council, Institute of Laboratory Animal Resources*. 46:280-293.
9. Coffman, R. L., A. Sher, and R. A. Seder. 2010. Vaccine adjuvants: putting innate immunity to work. *Immunity*. 33:492-503.
10. McKee, A. S., M. W. Munks, M. K. MacLeod, C. J. Fleenor, N. Van Rooijen, J. W. Kappler, and P. Marrack. 2009. Alum induces innate immune responses through macrophage and mast cell sensors, but these sensors are not required for alum to act as an adjuvant for specific immunity. *Journal of immunology (Baltimore, Md. : 1950)*. 183:4403-4414.
11. Eisenbarth, S. C., O. R. Colegio, W. O'Connor, F. S. Sutterwala, and R. A. Flavell. 2008. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. *Nature*. 453:1122-1126.
12. Kool, M., V. Petrilli, T. De Smedt, A. Rolaz, H. Hammad, M. van Nimwegen, I. M. Bergen, R. Castillo, B. N. Lambrecht, and J. Tschopp. 2008. Cutting edge: alum adjuvant stimulates inflammatory dendritic cells through activation of the NALP3 inflammasome. *Journal of immunology (Baltimore, Md. : 1950)*. 181:3755-3759.
13. Higgins, S. C. and K. H. Mills. 2010. TLR, NLR Agonists, and Other Immune Modulators as Infectious Disease Vaccine Adjuvants. *Current infectious disease reports*. 12:4-12.

14. Steinman, R. M. 2012. Decisions about dendritic cells: past, present, and future. *Annual review of immunology*. 30:1-22.
15. Eriksson, A. and N. Lycke. 2003. The CTA1-DD vaccine adjuvant binds to human B cells and potentiates their T cell stimulating ability. *Vaccine*. 22:185-193.
16. Campbell, P. A., C. Schuffler, and G. E. Rodriguez. 1976. Listeria cell wall fraction: a B cell adjuvant. *J Immunol*. 116:590-594.
17. Fevrier, M., J. L. Birrien, C. Leclerc, L. Chedid, and P. Liacopoulos. 1978. The macrophage, target cell of the synthetic adjuvant muramyl dipeptide. *European journal of immunology*. 8:558-562.
18. Kim, S., S. Lalani, V. V. Parekh, L. Wu, and L. Van Kaer. 2008. Glycolipid ligands of invariant natural killer T cells as vaccine adjuvants. *Expert Rev Vaccines*. 7:1519-1532.
19. Hall, L. J., S. Clare, and G. Dougan. 2010. NK cells influence both innate and adaptive immune responses after mucosal immunization with antigen and mucosal adjuvant. *Journal of immunology (Baltimore, Md. : 1950)*. 184:4327-4337.
20. Silk, J. D., I. F. Hermans, U. Gileadi, T. W. Chong, D. Shepherd, M. Salio, B. Mathew, R. R. Schmidt, S. J. Lunt, K. J. Williams, et al. 2004. Utilizing the adjuvant properties of CD1d-dependent NK T cells in T cell–mediated immunotherapy. *The Journal of Clinical Investigation*. 114:1800-1811.
21. McLachlan, J. B., C. P. Shelburne, J. P. Hart, S. V. Pizzo, R. Goyal, R. Brooking-Dixon, H. F. Staats, and S. N. Abraham. 2008. Mast cell activators: a new class of highly effective vaccine adjuvants. *Nat. Med.* 14:536-541.
22. Ribeiro, C. M. and V. E. Schijns. 2010. Immunology of vaccine adjuvants. *Methods Mol Biol*. 626:1-14.
23. Casella, C. R. and T. C. Mitchell. 2008. Putting endotoxin to work for us: monophosphoryl lipid A as a safe and effective vaccine adjuvant. *Cellular and molecular life sciences : CMLS*. 65:3231-3240.
24. Mata-Haro, V., C. Cekic, M. Martin, P. M. Chilton, C. R. Casella, and T. C. Mitchell. 2007. The vaccine adjuvant monophosphoryl lipid A as a TRIF-biased agonist of TLR4. *Science*. 316:1628-1632.
25. Bode, C., G. Zhao, F. Steinhagen, T. Kinjo, and D. M. Klinman. 2011. CpG DNA as a vaccine adjuvant. *Expert Rev Vaccines*. 10:499-511.
26. Mizel, S. B. and J. T. Bates. 2010. Flagellin as an adjuvant: cellular mechanisms and potential. *J Immunol*. 185:5677-5682.
27. Svennerholm, A. M. 2011. From cholera to enterotoxigenic Escherichia coli (ETEC) vaccine development. *Indian J. Med. Res.* 133:188-196.
28. Howard-Jones, N. 1984. Robert Koch and the cholera vibrio: a centenary. *Br Med J (Clin Res Ed)*. 288:379-381.
29. Zhang, R. G., D. L. Scott, M. L. Westbrook, S. Nance, B. D. Spangler, G. G. Shipley, and E. M. Westbrook. 1995. The three-dimensional crystal structure of cholera toxin. *J Mol Biol*. 251:563-573.

30. Sanchez, J. and J. Holmgren. 2011. Cholera toxin - a foe & a friend. *Indian J Med Res*. 133:153-163.
31. Tomasi, M., A. Battistini, A. Araco, L. G. Roda, and G. D'Agnolo. 1979. The role of the reactive disulfide bond in the interaction of cholera-toxin functional regions. *Eur J Biochem*. 93:621-627.
32. Sears, S. D., K. Richardson, C. Young, C. D. Parker, and M. M. Levine. 1984. Evaluation of the human immune response to outer membrane proteins of Vibrio cholerae. *Infection and immunity*. 44:439-444.
33. Fujihashi, K., T. Koga, F. W. van Ginkel, Y. Hagiwara, and J. R. McGhee. 2002. A dilemma for mucosal vaccination: efficacy versus toxicity using enterotoxin-based adjuvants. *Vaccine*. 20:2431-2438.
34. Dickinson, B. L. and J. D. Clements. 1995. Dissociation of Escherichia coli heat-labile enterotoxin adjuvanticity from ADP-ribosyltransferase activity. *Infection and immunity*. 63:1617-1623.
35. Giuliani, M. M., G. Del Giudice, V. Giannelli, G. Dougan, G. Douce, R. Rappuoli, and M. Pizza. 1998. Mucosal adjuvanticity and immunogenicity of LTR72, a novel mutant of Escherichia coli heat-labile enterotoxin with partial knockout of ADP-ribosyltransferase activity. *The Journal of experimental medicine*. 187:1123-1132.
36. Hagiwara, Y., Y. I. Kawamura, K. Kataoka, B. Rahima, R. J. Jackson, K. Komase, T. Dohi, P. N. Boyaka, Y. Takeda, H. Kiyono, et al. 2006. A second generation of double mutant cholera toxin adjuvants: enhanced immunity without intracellular trafficking. *Journal of immunology (Baltimore, Md. : 1950)*. 177:3045-3054.
37. Lycke, N. 2005. From toxin to adjuvant: basic mechanisms for the control of mucosal IgA immunity and tolerance. *Immunol. Lett.* 97:193-198.
38. Agren, L. C., L. Ekman, B. Lowenadler, and N. Y. Lycke. 1997. Genetically engineered nontoxic vaccine adjuvant that combines B cell targeting with immunomodulation by cholera toxin A1 subunit. *Journal of immunology (Baltimore, Md. : 1950)*. 158:3936-3946.
39. Agren, L., B. Lowenadler, and N. Lycke. 1998. A novel concept in mucosal adjuvanticity: the CTA1-DD adjuvant is a B cell-targeted fusion protein that incorporates the enzymatically active cholera toxin A1 subunit. *Immunol Cell Biol*. 76:280-287.
40. Eriksson, A. M., K. M. Schon, and N. Y. Lycke. 2004. The cholera toxin-derived CTA1-DD vaccine adjuvant administered intranasally does not cause inflammation or accumulate in the nervous tissues. *J. Immunol.* 173:3310-3319.
41. Cunningham, K. A., A. J. Carey, N. Lycke, P. Timms, and K. W. Beagley. 2009. CTA1-DD is an effective adjuvant for targeting anti-chlamydial immunity to the murine genital mucosa. *J Reprod Immunol*. 81:34-38.
42. Eliasson, D. G., K. El Bakkouri, K. Schon, A. Ramne, E. Festjens, B. Lowenadler, W. Fiers, X. Saelens, and N. Lycke. 2008. CTA1-M2e-DD: a novel mucosal adjuvant targeted influenza vaccine. *Vaccine*. 26:1243-1252.

43. Andersen, C. S., J. Dietrich, E. M. Agger, N. Y. Lycke, K. Lovgren, and P. Andersen. 2007. The combined CTA1-DD/ISCOMs vector is an effective intranasal adjuvant for boosting prior Mycobacterium bovis BCG immunity to Mycobacterium tuberculosis. *Infect. Immun.* 75:408-416.

44. Sundling, C., K. Schon, A. Morner, M. N. Forsell, R. T. Wyatt, R. Thorstensson, G. B. Karlsson Hedestam, and N. Y. Lycke. 2008. CTA1-DD adjuvant promotes strong immunity against human immunodeficiency virus type 1 envelope glycoproteins following mucosal immunization. *J. Gen. Virol.* 89:2954-2964.

45. Agren, L. C., L. Ekman, B. Lowenadler, J. G. Nedrud, and N. Y. Lycke. 1999. Adjuvanticity of the cholera toxin A1-based gene fusion protein, CTA1-DD, is critically dependent on the ADP-ribosyltransferase and Ig-binding activity. *Journal of immunology (Baltimore, Md. : 1950)*. 162:2432-2440.

46. Agren, L., E. Sverremark, L. Ekman, K. Schon, B. Lowenadler, C. Fernandez, and N. Lycke. 2000. The ADP-ribosylating CTA1-DD adjuvant enhances T cell-dependent and independent responses by direct action on B cells involving anti-apoptotic Bcl-2- and germinal center-promoting effects. *J. Immunol.* 164:6276-6286.

47. Bemark, M., P. Bergqvist, A. Stensson, A. Holmberg, J. Mattsson, and N. Y. Lycke. 2011. A unique role of the cholera toxin A1-DD adjuvant for long-term plasma and memory B cell development. *Journal of immunology (Baltimore, Md. : 1950)*. 186:1399-1410.

48. Mattsson, J., U. Yrlid, A. Stensson, K. Schon, M. C. Karlsson, J. V. Ravetch, and N. Y. Lycke. 2011. Complement activation and complement receptors on follicular dendritic cells are critical for the function of a targeted adjuvant. *Journal of immunology (Baltimore, Md. : 1950)*. 187:3641-3652.

49. Agren, L. C., L. Ekman, B. Lowenadler, J. G. Nedrud, and N. Y. Lycke. 1999. Adjuvanticity of the cholera toxin A1-based gene fusion protein, CTA1-DD, is critically dependent on the ADP-ribosyltransferase and Ig-binding activity. *J. Immunol.* 162:2432-2440.

50. Hasselberg, A., L. Ekman, L. F. Yrlid, K. Schon, and N. Y. Lycke. 2010. ADP-ribosylation controls the outcome of tolerance or enhanced priming following mucosal immunization. *Journal of immunology (Baltimore, Md. : 1950)*. 184:2776-2784.

51. Hasselberg, A., K. Schon, A. Tarkowski, and N. Lycke. 2009. Role of CTA1R7K-COL-DD as a novel therapeutic mucosal tolerance-inducing vector for treatment of collagen-induced arthritis. *Arthritis Rheum*. 60:1672-1682.

52. Galli, S. J., M. Tsai, and A. M. Piliponsky. 2008. The development of allergic inflammation. *Nature*. 454:445-454.

53. Wedemeyer, J., M. Tsai, and S. J. Galli. 2000. Roles of mast cells and basophils in innate and acquired immunity. *Curr Opin Immunol*. 12:624-631.

54. Dvorak, A. M., R. A. Seder, W. E. Paul, E. S. Morgan, and S. J. Galli. 1994. Effects of interleukin-3 with or without the c-kit ligand, stem cell factor, on the survival and cytoplasmic granule formation of mouse basophils and mast cells in vitro. *Am J Pathol*. 144:160-170.
55. Andersson, C. K., M. Mori, L. Bjermer, C. G. Lofdahl, and J. S. Erjefalt. 2010. Alterations in lung mast cell populations in patients with chronic obstructive pulmonary disease. *American journal of respiratory and critical care medicine*. 181:206-217.
56. Andersson, C. K., M. Mori, L. Bjermer, C. G. Lofdahl, and J. S. Erjefalt. 2009. Novel site-specific mast cell subpopulations in the human lung. *Thorax*. 64:297-305.
57. Galli, S. J., J. Kalesnikoff, M. A. Grimbaldeston, A. M. Piliponsky, C. M. Williams, and M. Tsai. 2005. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annual review of immunology*. 23:749-786.
58. Abraham, S. N. and A. L. St John. 2010. Mast cell-orchestrated immunity to pathogens. *Nat. Rev. Immunol.* 10:440-452.
59. Moon, T. C., C. D. St Laurent, K. E. Morris, C. Marcet, T. Yoshimura, Y. Sekar, and A. D. Befus. 2010. Advances in mast cell biology: new understanding of heterogeneity and function. *Mucosal Immunol*. 3:111-128.
60. Rao, K. N. and M. A. Brown. 2008. Mast cells: multifaceted immune cells with diverse roles in health and disease. *Ann N Y Acad Sci*. 1143:83-104.
61. Galli, S. J. and M. Tsai. 2008. Mast cells: versatile regulators of inflammation, tissue remodeling, host defense and homeostasis. *J. Dermatol. Sci.* 49:7-19.
62. Georgin-Lavialle, S., L. Lhermitte, P. Dubreuil, M. O. Chandesris, O. Hermine, and G. Damaj. 2013. Mast cell leukemia. *Blood*. 121:1285-1295.
63. Georgin-Lavialle, S., C. Aguilar, R. Guieze, L. Lhermitte, J. Bruneau, S. Fraitag, D. Canioni, M. O. Chandesris, F. Suarez, C. Grandpeix-Guyodo, et al. 2013. Mast cell sarcoma: a rare and aggressive entity--report of two cases and review of the literature. *J Clin Oncol*. 31:e90-97.
64. Amagai, Y., A. Tanaka, A. Matsuda, K. Jung, K. Ohmori, and H. Matsuda. 2013. Stem cell factor contributes to tumorigenesis of mast cells via an autocrine/paracrine mechanism. *J Leukoc Biol*. 93:245-250.
65. Khazaie, K., N. R. Blatner, M. W. Khan, F. Gounari, E. Gounaris, K. Dennis, A. Bonertz, F. N. Tsai, M. J. Strouch, E. Cheon, et al. 2011. The significant role of mast cells in cancer. *Cancer Metastasis Rev*. 30:45-60.
66. Blatner, N. R., A. Bonertz, P. Beckhove, E. C. Cheon, S. B. Krantz, M. Strouch, J. Weitz, M. Koch, A. L. Halverson, D. J. Bentrem, et al. 2010. In colorectal cancer mast cells contribute to systemic regulatory T-cell dysfunction. *Proc Natl Acad Sci U S A*. 107:6430-6435.
67. Rabenhorst, A., M. Schlaak, L. C. Heukamp, A. Forster, S. Theurich, M. von Bergwelt-Baildon, R. Buttner, P. Kurschat, C. Mauch, A. Roers, et al. 2012. Mast cells play a protumorigenic role in primary cutaneous lymphoma. *Blood*. 120:2042-2054.

68. Khan, M. W., A. Keshavarzian, E. Gounaris, J. E. Melson, E. Cheon, N. R. Blatner, Z. E. Chen, F. N. Tsai, G. Lee, H. Ryu, et al. 2013. PI3K/AKT signaling is essential for communication between tissue infiltrating mast cells, macrophages, and epithelial cells in colitis-induced cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research*.
69. Levick, S. P., G. C. Melendez, E. Plante, J. L. McLarty, G. L. Brower, and J. S. Janicki. 2011. Cardiac mast cells: the centrepiece in adverse myocardial remodelling. *Cardiovascular research*. 89:12-19.
70. Zhang, W., A. L. Chancey, H. P. Tzeng, Z. Zhou, K. J. Lavine, F. Gao, N. Sivasubramanian, P. M. Barger, and D. L. Mann. 2011. The development of myocardial fibrosis in transgenic mice with targeted overexpression of tumor necrosis factor requires mast cell-fibroblast interactions. *Circulation*. 124:2106-2116.
71. Guo, T., W. Q. Chen, C. Zhang, Y. X. Zhao, and Y. Zhang. 2009. Chymase activity is closely related with plaque vulnerability in a hamster model of atherosclerosis. *Atherosclerosis*. 207:59-67.
72. den Dekker, W. K., D. Tempel, I. Bot, E. A. Biessen, L. A. Joosten, M. G. Netea, J. W. van der Meer, C. Cheng, and H. J. Duckers. 2012. Mast cells induce vascular smooth muscle cell apoptosis via a toll-like receptor 4 activation pathway. *Arteriosclerosis, thrombosis, and vascular biology*. 32:1960-1969.
73. Sayed, B. A., A. Christy, M. R. Quirion, and M. A. Brown. 2008. The master switch: the role of mast cells in autoimmunity and tolerance. *Annual review of immunology*. 26:705-739.
74. Christy, A. L., M. E. Walker, M. J. Hessner, and M. A. Brown. 2013. Mast cell activation and neutrophil recruitment promotes early and robust inflammation in the meninges in EAE. *Journal of autoimmunity*. 42:50-61.
75. Secor, V. H., W. E. Secor, C. A. Gutekunst, and M. A. Brown. 2000. Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. *The Journal of experimental medicine*. 191:813-822.
76. Eklund, K. K. 2007. Mast cells in the pathogenesis of rheumatic diseases and as potential targets for anti-rheumatic therapy. *Immunol Rev*. 217:38-52.
77. Hueber, A. J., D. L. Asquith, A. M. Miller, J. Reilly, S. Kerr, J. Leipe, A. J. Melendez, and I. B. McInnes. 2010. Mast cells express IL-17A in rheumatoid arthritis synovium. *Journal of immunology (Baltimore, Md. : 1950)*. 184:3336-3340.
78. Sismanopoulos, N., D. A. Delivanis, D. Mavrommati, E. Hatziagelaki, P. Conti, and T. C. Theoharides. 2013. Do mast cells link obesity and asthma? *Allergy*. 68:8-15.
79. Liu, J., A. Divoux, J. Sun, J. Zhang, K. Clement, J. N. Glickman, G. K. Sukhova, P. J. Wolters, J. Du, C. Z. Gorgun, et al. 2009. Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. *Nature medicine*. 15:940-945.

80. Divoux, A., S. Moutel, C. Poitou, D. Lacasa, N. Veyrie, A. Aissat, M. Arock, M. Guerre-Millo, and K. Clement. 2012. Mast cells in human adipose tissue: link with morbid obesity, inflammatory status, and diabetes. *J Clin Endocrinol Metab*. 97:E1677-1685.
81. Rodewald, H. R. and T. B. Feyerabend. 2012. Widespread immunological functions of mast cells: fact or fiction? *Immunity*. 37:13-24.
82. Chan, C. Y., A. L. St John, and S. N. Abraham. 2013. Mast cell interleukin-10 drives localized tolerance in chronic bladder infection. *Immunity*. 38:349-359.
83. Grimbaldeston, M. A., S. Nakae, J. Kalesnikoff, M. Tsai, and S. J. Galli. 2007. Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. *Nature immunology*. 8:1095-1104.
84. Couper, K. N., D. G. Blount, and E. M. Riley. 2008. IL-10: the master regulator of immunity to infection. *Journal of immunology (Baltimore, Md. : 1950)*. 180:5771-5777.
85. Schmidt-Weber, C. B. and K. Blaser. 2004. Regulation and role of transforming growth factor-beta in immune tolerance induction and inflammation. *Curr Opin Immunol*. 16:709-716.
86. Chacon-Salinas, R., A. Y. Limon-Flores, A. D. Chavez-Blanco, A. Gonzalez-Estrada, and S. E. Ullrich. 2011. Mast cell-derived IL-10 suppresses germinal center formation by affecting T follicular helper cell function. *Journal of immunology (Baltimore, Md. : 1950)*. 186:25-31.
87. Lu, L. F., E. F. Lind, D. C. Gondek, K. A. Bennett, M. W. Gleeson, K. Pino-Lagos, Z. A. Scott, A. J. Coyle, J. L. Reed, J. Van Snick, et al. 2006. Mast cells are essential intermediaries in regulatory T-cell tolerance. *Nature*. 442:997-1002.
88. Nakano, T., C. Y. Lai, S. Goto, L. W. Hsu, S. Kawamoto, K. Ono, K. D. Chen, C. C. Lin, K. W. Chiu, C. C. Wang, et al. 2012. Immunological and regenerative aspects of hepatic mast cells in liver allograft rejection and tolerance. *PLoS One*. 7:e37202.
89. de Vries, V. C., R. Elgueta, D. M. Lee, and R. J. Noelle. 2010. Mast cell protease 6 is required for allograft tolerance. *Transplant Proc*. 42:2759-2762.
90. Waern, I., S. Jonasson, J. Hjoberg, A. Bucht, M. Abrink, G. Pejler, and S. Wernersson. 2009. Mouse mast cell protease 4 is the major chymase in murine airways and has a protective role in allergic airway inflammation. *J Immunol*. 183:6369-6376.
91. Piliponsky, A. M., C. C. Chen, E. J. Rios, P. M. Treuting, A. Lahiri, M. Abrink, G. Pejler, M. Tsai, and S. J. Galli. 2012. The chymase mouse mast cell protease 4 degrades TNF, limits inflammation, and promotes survival in a model of sepsis. *Am J Pathol*. 181:875-886.
92. Hershko, A. Y., R. Suzuki, N. Charles, D. Alvarez-Errico, J. L. Sargent, A. Laurence, and J. Rivera. 2011. Mast cell interleukin-2 production

contributes to suppression of chronic allergic dermatitis. *Immunity*. 35:562-571.

93. Enerback, L. 1987. Mucosal mast cells in the rat and in man. *Int Arch Allergy Appl Immunol*. 82:249-255.
94. Kitamura, Y. 1989. Heterogeneity of mast cells and phenotypic change between subpopulations. *Annu. Rev. Immunol.* 7:59-76.
95. Enerback, L. 1966. Mast cells in rat gastrointestinal mucosa. 2. Dye-binding and metachromatic properties. *Acta Pathol Microbiol Scand*. 66:303-312.
96. Vliagoftis, H. and A. D. Befus. 2005. Rapidly changing perspectives about mast cells at mucosal surfaces. *Immunol Rev*. 206:190-203.
97. Gersch, C., O. Dewald, M. Zoerlein, L. H. Michael, M. L. Entman, and N. G. Frangogiannis. 2002. Mast cells and macrophages in normal C57/BL/6 mice. *Histochem. Cell Biol.* 118:41-49.
98. Irani, A. M., T. R. Bradford, C. L. Kepley, N. M. Schechter, and L. B. Schwartz. 1989. Detection of MCT and MCTC types of human mast cells by immunohistochemistry using new monoclonal anti-tryptase and anti-chymase antibodies. *J Histochem Cytochem*. 37:1509-1515.
99. Toru, H., M. Eguchi, R. Matsumoto, M. Yanagida, J. Yata, and T. Nakahata. 1998. Interleukin-4 promotes the development of tryptase and chymase double-positive human mast cells accompanied by cell maturation. *Blood*. 91:187-195.
100. Garman, S. C., B. A. Wurzburg, S. S. Tarchevskaya, J. P. Kinet, and T. S. Jardetzky. 2000. Structure of the Fc fragment of human IgE bound to its high-affinity receptor Fc epsilonRI alpha. *Nature*. 406:259-266.
101. Kawakami, T. and S. J. Galli. 2002. Regulation of mast-cell and basophil function and survival by IgE. *Nat. Rev. Immunol.* 2:773-786.
102. D'Amato, G., M. Perticone, E. Bucchioni, A. Salzillo, M. D'Amato, and G. Liccardi. 2010. Treating moderate-to-severe allergic asthma with anti-IgE monoclonal antibody (omalizumab). An update. *European annals of allergy and clinical immunology*. 42:135-140.
103. Buhl, R. 2005. Anti-IgE antibodies for the treatment of asthma. *Current opinion in pulmonary medicine*. 11:27-34.
104. Busse, W. W., W. J. Morgan, P. J. Gergen, H. E. Mitchell, J. E. Gern, A. H. Liu, R. S. Gruchalla, M. Kattan, S. J. Teach, J. A. Pongracic, et al. 2011. Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. *N Engl J Med*. 364:1005-1015.
105. Kim, B., A. Eggel, S. S. Tarchevskaya, M. Vogel, H. Prinz, and T. S. Jardetzky. 2012. Accelerated disassembly of IgE-receptor complexes by a disruptive macromolecular inhibitor. *Nature*. 491:613-617.
106. Malbec, O. and M. Daeron. 2007. The mast cell IgG receptors and their roles in tissue inflammation. *Immunol. Rev.* 217:206-221.
107. Tkaczyk, C., Y. Okayama, D. D. Metcalfe, and A. M. Gilfillan. 2004. Fcgamma receptors on mast cells: activatory and inhibitory regulation of mediator release. *Int. Arch. Allergy Immunol.* 133:305-315.

108. Jonsson, F. and M. Daeron. 2012. Mast cells and company. *Front Immunol*. 3:16.
109. Ovary, Z., H. Fudenberg, and H. G. Kunkel. 1960. Anuphylactic reactions in the skin of the guinea pig with high and low molecular weight antibodies and gamma globulins. *The Journal of experimental medicine*. 112:953-961.
110. Nimmerjahn, F. and J. V. Ravetch. 2008. Fcgamma receptors as regulators of immune responses. *Nat. Rev. Immunol.* 8:34-47.
111. Bruhns, P. 2012. Properties of mouse and human IgG receptors and their contribution to disease models. *Blood*. 119:5640-5649.
112. Smith, K. G. C. and M. R. Clatworthy. 2010. FcgammaRIIB in autoimmunity and infection: evolutionary and therapeutic implications. *Nat. Rev. Immunol.* 10:328-343.
113. Park-Min, K. H., N. V. Serbina, W. Yang, X. Ma, G. Krystal, B. G. Neel, S. L. Nutt, X. Hu, and L. B. Ivashkiv. 2007. FcgammaRIII-dependent inhibition of interferon-gamma responses mediates suppressive effects of intravenous immune globulin. *Immunity*. 26:67-78.
114. Aloulou, M., S. Ben Mkaddem, M. Biarnes-Pelicot, T. Boussetta, H. Souchet, E. Rossato, M. Benhamou, B. Crestani, Z. Zhu, U. Blank, et al. 2012. IgG1 and IVIg induce inhibitory ITAM signaling through FcgammaRIII controlling inflammatory responses. *Blood*. 119:3084-3096.
115. Okayama, Y., A. S. Kirshenbaum, and D. D. Metcalfe. 2000. Expression of a functional high-affinity IgG receptor, Fc gamma RI, on human mast cells: Up-regulation by IFN-gamma. *J. Immunol.* 164:4332-4339.
116. Woolhiser, M. R., Y. Okayama, A. M. Gilfillan, and D. D. Metcalfe. 2001. IgG-dependent activation of human mast cells following up-regulation of FcgammaRI by IFN-gamma. *Eur. J. Immunol.* 31:3298-3307.
117. Tkaczyk, C., Y. Okayama, M. R. Woolhiser, D. D. Hagaman, A. M. Gilfillan, and D. D. Metcalfe. 2002. Activation of human mast cells through the high affinity IgG receptor. *Mol. Immunol.* 38:1289-1293.
118. Zhao, W., C. L. Kepley, P. A. Morel, L. M. Okumoto, Y. Fukuoka, and L. B. Schwartz. 2006. Fc gamma RIIa, not Fc gamma RIIb, is constitutively and functionally expressed on skin-derived human mast cells. *J. Immunol.* 177:694-701.
119. Kepley, C. L., S. Taghavi, G. Mackay, D. Zhu, P. A. Morel, K. Zhang, J. J. Ryan, L. S. Satin, M. Zhang, P. P. Pandolfi, et al. 2004. Co-aggregation of FcgammaRII with FcepsilonRI on human mast cells inhibits antigen-induced secretion and involves SHIP-Grb2-Dok complexes. *J. Biol. Chem.* 279:35139-35149.
120. Okayama, Y., D. D. Hagaman, M. Woolhiser, and D. D. Metcalfe. 2001. Further characterization of FcgammaRII and FcgammaRIII expression by cultured human mast cells. *Int. Arch. Allergy Immunol.* 124:155-157.
121. Benhamou, M., C. Bonnerot, W. H. Fridman, and M. Daeron. 1990. Molecular heterogeneity of murine mast cell Fc gamma receptors. *Journal of immunology (Baltimore, Md. : 1950)*. 144:3071-3077.

122. Katz, H. R., J. P. Arm, A. C. Benson, and K. F. Austen. 1990. Maturation-related changes in the expression of Fc gamma RII and Fc gamma RIII on mouse mast cells derived in vitro and in vivo. *Journal of immunology (Baltimore, Md. : 1950)*. 145:3412-3417.

123. Vaz, N. M. and Z. Ovary. 1968. Passive anaphylaxis in mice with gamma-G antibodies. 3. Release of histamine from mast cells by homologous antibodies. *Journal of immunology (Baltimore, Md. : 1950)*. 100:1014-1019.

124. Katz, H. R., M. B. Raizman, C. S. Gartner, H. C. Scott, A. C. Benson, and K. F. Austen. 1992. Secretory granule mediator release and generation of oxidative metabolites of arachidonic acid via Fc-IgG receptor bridging in mouse mast cells. *Journal of immunology (Baltimore, Md. : 1950)*. 148:868-871.

125. Lobell, R. B., K. F. Austen, and H. R. Katz. 1994. Fc gamma R-mediated endocytosis and expression of cell surface Fc gamma RIIb1 and Fc gamma RIIb2 by mouse bone marrow culture-derived progenitor mast cells. *J. Immunol.* 152:811-818.

126. Daeron, M., O. Malbec, S. Latour, M. Arock, and W. H. Fridman. 1995. Regulation of high-affinity IgE receptor-mediated mast cell activation by murine low-affinity IgG receptors. *J Clin Invest*. 95:577-585.

127. Takai, T., M. Ono, M. Hikida, H. Ohmori, and J. V. Ravetch. 1996. Augmented humoral and anaphylactic responses in Fc gamma RII-deficient mice. *Nature*. 379:346-349.

128. Malbec, O., K. Roget, C. Schiffer, B. Iannascoli, A. R. Dumas, M. Arock, and M. Daeron. 2007. Peritoneal cell-derived mast cells: an in vitro model of mature serosal-type mouse mast cells. *Journal of immunology (Baltimore, Md. : 1950)*. 178:6465-6475.

129. Allen, L. C., C. L. Kepley, A. Saxon, and K. Zhang. 2007. Modifications to an Fcgamma-Fcvarepsilon fusion protein alter its effectiveness in the inhibition of FcvarepsilonRI-mediated functions. *J Allergy Clin Immunol*. 120:462-468.

130. Zhu, D., C. L. Kepley, M. Zhang, K. Zhang, and A. Saxon. 2002. A novel human immunoglobulin Fc gamma Fc epsilon bifunctional fusion protein inhibits Fc epsilon RI-mediated degranulation. *Nat. Med.* 8:518-521.

131. Tam, S. W., S. Demissie, D. Thomas, and M. Daeron. 2004. A bispecific antibody against human IgE and human FcgammaRII that inhibits antigen-induced histamine release by human mast cells and basophils. *Allergy*. 59:772-780.

132. Xiang, Z., A. A. Ahmed, C. Moller, K. Nakayama, S. Hatakeyama, and G. Nilsson. 2001. Essential role of the prosurvival bcl-2 homologue A1 in mast cell survival after allergic activation. *J. Exp. Med.* 194:1561-1569.

133. Yoshikawa, H., Y. Nakajima, and K. Tasaka. 1999. Glucocorticoid suppresses autocrine survival of mast cells by inhibiting IL-4 production and ICAM-1 expression. *J. Immunol.* 162:6162-6170.

134. Kobayasi, T. and G. Asboe-Hansen. 1969. Degranulation and regranulation of human mast cells. An electron microscopic study of the whealing reaction in urticaria pigmentosa. *Acta Derm Venereol*. 49:369-381.
135. Xiang, Z., M. Block, C. Lofman, and G. Nilsson. 2001. IgE-mediated mast cell degranulation and recovery monitored by time-lapse photography. *J. Allergy Clin. Immunol.* 108:116-121.
136. Dvorak, A. M., R. P. Schleimer, E. S. Schulman, and L. M. Lichtenstein. 1986. Human mast cells use conservation and condensation mechanisms during recovery from degranulation. In vitro studies with mast cells purified from human lungs. *Lab Invest*. 54:663-678.
137. Ekoff, M., A. Strasser, and G. Nilsson. 2007. FcepsilonRI aggregation promotes survival of connective tissue-like mast cells but not mucosal-like mast cells. *J. Immunol.* 178:4177-4183.
138. Green, D. R. and D. W. Scott. 1994. Activation-induced apoptosis in lymphocytes. *Curr Opin Immunol*. 6:476-487.
139. Elmore, S. 2007. Apoptosis: a review of programmed cell death. *Toxicologic pathology*. 35:495-516.
140. Yin, X. M. 2000. Signal transduction mediated by Bid, a pro-death Bcl-2 family proteins, connects the death receptor and mitochondria apoptosis pathways. *Cell research*. 10:161-167.
141. Lorenzo, H. K. and S. A. Susin. 2004. Mitochondrial effectors in caspase-independent cell death. *FEBS letters*. 557:14-20.
142. Liu, Y. and D. M. Templeton. 2008. Initiation of caspase-independent death in mouse mesangial cells by Cd2+: involvement of p38 kinase and CaMK-II. *Journal of cellular physiology*. 217:307-318.
143. Yoshikawa, H. and K. Tasaka. 2003. Caspase-dependent and -independent apoptosis of mast cells induced by withdrawal of IL-3 is prevented by Toll-like receptor 4-mediated lipopolysaccharide stimulation. *European journal of immunology*. 33:2149-2159.
144. Mekori, Y. A., C. K. Oh, and D. D. Metcalfe. 1993. IL-3-dependent murine mast cells undergo apoptosis on removal of IL-3. Prevention of apoptosis by c-kit ligand. *Journal of immunology (Baltimore, Md. : 1950)*. 151:3775-3784.
145. Ekoff, M., T. Kaufmann, M. Engstrom, N. Motoyama, A. Villunger, J. I. Jonsson, A. Strasser, and G. Nilsson. 2007. The BH3-only protein Puma plays an essential role in cytokine deprivation induced apoptosis of mast cells. *Blood*. 110:3209-3217.
146. Bailey, D. P., M. Kashyap, L. A. Bouton, P. J. Murray, and J. J. Ryan. 2006. Interleukin-10 induces apoptosis in developing mast cells and macrophages. *J Leukoc Biol*. 80:581-589.
147. Mann-Chandler, M. N., M. Kashyap, H. V. Wright, F. Norozian, B. O. Barnstein, S. Gingras, E. Parganas, and J. J. Ryan. 2005. IFN-gamma induces apoptosis in developing mast cells. *Journal of immunology (Baltimore, Md. : 1950)*. 175:3000-3005.

148. Norozian, F., M. Kashyap, C. D. Ramirez, N. Patel, C. L. Kepley, B. O. Barnstein, and J. J. Ryan. 2006. TGFbeta1 induces mast cell apoptosis. *Exp Hematol*. 34:579-587.
149. Mekori, Y. A. and D. D. Metcalfe. 1994. Transforming growth factor-beta prevents stem cell factor-mediated rescue of mast cells from apoptosis after IL-3 deprivation. *Journal of immunology (Baltimore, Md. : 1950)*. 153:2194-2203.
150. Godfrey, H. P., C. Ilardi, W. Engber, and F. M. Graziano. 1984. Quantitation of human synovial mast cells in rheumatoid arthritis and other rheumatic diseases. *Arthritis Rheum*. 27:852-856.
151. Hussain, S. S. and J. M. Hopkinson. 1995. Mast cells in aural polyps: a preliminary report. *The Journal of laryngology and otology*. 109:491-494.
152. Abonia, J. P., J. Hallgren, T. Jones, T. Shi, Y. Xu, P. Koni, R. A. Flavell, J. A. Boyce, K. F. Austen, and M. F. Gurish. 2006. Alpha-4 integrins and VCAM-1, but not MAdCAM-1, are essential for recruitment of mast cell progenitors to the inflamed lung. *Blood*. 108:1588-1594.
153. Nagata, S. 2010. Apoptosis and autoimmune diseases. *Ann N Y Acad Sci*. 1209:10-16.
154. Lowe, S. W. and A. W. Lin. 2000. Apoptosis in cancer. *Carcinogenesis*. 21:485-495.
155. Ma, Z., J. P. Tovar, K. Y. Kwong, and D. Paek. 2010. Pimecrolimus induces apoptosis of mast cells in a murine model of cutaneous mastocytosis. *Int Arch Allergy Immunol*. 153:413-418.
156. Sheen, C. H., R. P. Schleimer, and M. Kulka. 2007. Codeine induces human mast cell chemokine and cytokine production: involvement of G-protein activation. *Allergy*. 62:532-538.
157. Blunk, J. A., M. Schmelz, S. Zeck, P. Skov, R. Likar, and W. Koppert. 2004. Opioid-induced mast cell activation and vascular responses is not mediated by mu-opioid receptors: an in vivo microdialysis study in human skin. *Anesthesia and analgesia*. 98:364-370, table of contents.
158. Bernstein, I. L., S. C. Siegel, M. L. Brandon, E. B. Brown, R. R. Evans, A. R. Feinberg, S. Friedlaender, R. A. Krumholz, R. A. Hadley, N. I. Handelman, et al. 1972. A controlled study of cromolyn sodium sponsored by the Drug Committee of the American Academy of Allergy. *J Allergy Clin Immunol*. 50:235-245.
159. Oka, T., J. Kalesnikoff, P. Starkl, M. Tsai, and S. J. Galli. 2012. Evidence questioning cromolyn's effectiveness and selectivity as a 'mast cell stabilizer' in mice. *Laboratory investigation; a journal of technical methods and pathology*. 92:1472-1482.
160. Weng, Z., B. Zhang, S. Asadi, N. Sismanopoulos, A. Butcher, X. Fu, A. Katsarou-Katsari, C. Antoniou, and T. C. Theoharides. 2012. Quercetin is more effective than cromolyn in blocking human mast cell cytokine release and inhibits contact dermatitis and photosensitivity in humans. *PLoS One*. 7:e33805.

161. Kempuraj, D., B. Madhappan, S. Christodoulou, W. Boucher, J. Cao, N. Papadopoulou, C. L. Cetrulo, and T. C. Theoharides. 2005. Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. *Br J Pharmacol*. 145:934-944.

162. Theoharides, T. C., P. Patra, W. Boucher, R. Letourneau, D. Kempuraj, G. Chiang, S. Jeudy, L. Hesse, and A. Athanasiou. 2000. Chondroitin sulphate inhibits connective tissue mast cells. *Br J Pharmacol*. 131:1039-1049.

163. de Jonge, W. J., F. O. The, D. van der Coelen, R. J. Bennink, P. H. Reitsma, S. J. van Deventer, R. M. van den Wijngaard, and G. E. Boeckxstaens. 2004. Mast cell degranulation during abdominal surgery initiates postoperative ileus in mice. *Gastroenterology*. 127:535-545.

164. Ohta, Y., T. Kobayashi, K. Nishida, and I. Ishiguro. 1997. Relationship between changes of active oxygen metabolism and blood flow and formation, progression, and recovery of lesions is gastric mucosa of rats with a single treatment of compound 48/80, a mast cell degranulator. *Dig Dis Sci*. 42:1221-1232.

165. Joffre, O. P., E. Segura, A. Savina, and S. Amigorena. 2012. Cross-presentation by dendritic cells. *Nature reviews. Immunology*. 12:557-569.

166. Spicer, S. S., J. A. Simson, and J. E. Farrington. 1975. Mast cell phagocytosis of red blood cells. *Am J Pathol*. 80:481-498.

167. Malaviya, R., E. A. Ross, J. I. MacGregor, T. Ikeda, J. R. Little, B. A. Jakschik, and S. N. Abraham. 1994. Mast cell phagocytosis of FimH-expressing enterobacteria. *Journal of immunology (Baltimore, Md. : 1950)*. 152:1907-1914.

168. Della Rovere, F., A. Granata, M. Monaco, and G. Basile. 2009. Phagocytosis of cancer cells by mast cells in breast cancer. *Anticancer Res*. 29:3157-3161.

169. Sher, A., A. Hein, G. Moser, and J. P. Caulfield. 1979. Complement receptors promote the phagocytosis of bacteria by rat peritoneal mast cells. *Lab Invest*. 41:490-499.

170. Kambayashi, T., J. D. Baranski, R. G. Baker, T. Zou, E. J. Allenspach, J. E. Shoag, P. L. Jones, and G. A. Koretzky. 2008. Indirect involvement of allergen-captured mast cells in antigen presentation. *Blood*. 111:1489-1496.

171. Kambayashi, T., E. J. Allenspach, J. T. Chang, T. Zou, J. E. Shoag, S. L. Reiner, A. J. Caton, and G. A. Koretzky. 2009. Inducible MHC class II expression by mast cells supports effector and regulatory T cell activation. *Journal of immunology (Baltimore, Md. : 1950)*. 182:4686-4695.

172. Gaudenzio, N., N. Espagnolle, L. T. Mars, R. Liblau, S. Valitutti, and E. Espinosa. 2009. Cell-cell cooperation at the T helper cell/mast cell immunological synapse. *Blood*. 114:4979-4988.

173. Poncet, P., M. Arock, and B. David. 1999. MHC class II-dependent activation of CD4+ T cell hybridomas by human mast cells through superantigen presentation. *J Leukoc Biol*. 66:105-112.

174. Galli, S. J., S. Nakae, and M. Tsai. 2005. Mast cells in the development of adaptive immune responses. *Nature immunology*. 6:135-142.
175. Nakae, S., H. Suto, M. Iikura, M. Kakurai, J. D. Sedgwick, M. Tsai, and S. J. Galli. 2006. Mast cells enhance T cell activation: importance of mast cell costimulatory molecules and secreted TNF. *Journal of immunology (Baltimore, Md. : 1950)*. 176:2238-2248.
176. Stelekati, E., R. Bahri, O. D'Orlando, Z. Orinska, H. W. Mittrucker, R. Langenhaun, M. Glatzel, A. Bollinger, R. Paus, and S. Bulfone-Paus. 2009. Mast cell-mediated antigen presentation regulates CD8+ T cell effector functions. *Immunity*. 31:665-676.
177. Woodbury, R. G., H. R. Miller, J. F. Huntley, G. F. Newlands, A. C. Palliser, and D. Wakelin. 1984. Mucosal mast cells are functionally active during spontaneous expulsion of intestinal nematode infections in rat. *Nature*. 312:450-452.
178. Nawa, Y., M. Kiyota, M. Korenaga, and M. Kotani. 1985. Defective protective capacity of W/Wv mice against Strongyloides ratti infection and its reconstitution with bone marrow cells. *Parasite Immunol*. 7:429-438.
179. Caron, G., Y. Delneste, E. Roelandts, C. Duez, N. Herbault, G. Magistrelli, J. Y. Bonnefoy, J. Pestel, and P. Jeannin. 2001. Histamine induces CD86 expression and chemokine production by human immature dendritic cells. *Journal of immunology (Baltimore, Md. : 1950)*. 166:6000-6006.
180. Dawicki, W., D. W. Jawdat, N. Xu, and J. S. Marshall. 2010. Mast cells, histamine, and IL-6 regulate the selective influx of dendritic cell subsets into an inflamed lymph node. *Journal of immunology (Baltimore, Md. : 1950)*. 184:2116-2123.
181. Amaral, M. M., C. Davio, A. Ceballos, G. Salamone, C. Canones, J. Geffner, and M. Vermeulen. 2007. Histamine improves antigen uptake and cross-presentation by dendritic cells. *Journal of immunology (Baltimore, Md. : 1950)*. 179:3425-3433.
182. Suto, H., S. Nakae, M. Kakurai, J. D. Sedgwick, M. Tsai, and S. J. Galli. 2006. Mast cell-associated TNF promotes dendritic cell migration. *J. Immunol.* 176:4102-4112.
183. McLachlan, J. B., J. P. Hart, S. V. Pizzo, C. P. Shelburne, H. F. Staats, M. D. Gunn, and S. N. Abraham. 2003. Mast cell-derived tumor necrosis factor induces hypertrophy of draining lymph nodes during infection. *Nature immunology*. 4:1199-1205.
184. Jawdat, D. M., G. Rowden, and J. S. Marshall. 2006. Mast cells have a pivotal role in TNF-independent lymph node hypertrophy and the mobilization of Langerhans cells in response to bacterial peptidoglycan. *Journal of immunology (Baltimore, Md. : 1950)*. 177:1755-1762.
185. Suto, H., S. Nakae, M. Kakurai, J. D. Sedgwick, M. Tsai, and S. J. Galli. 2006. Mast cell-associated TNF promotes dendritic cell migration. *Journal of immunology (Baltimore, Md. : 1950)*. 176:4102-4112.
186. Shelburne, C. P., H. Nakano, A. L. St. John, C. Chan, J. B. McLachlan, M. D. Gunn, H. F. Staats, and S. N. Abraham. 2009. Mast Cells Augment

Adaptive Immunity by Orchestrating Dendritic Cell Trafficking through Infected Tissues. *Cell Host & Microbe*. 6:331-342.

187. Heib, V., M. Becker, T. Warger, G. Rechtsteiner, C. Tertilt, M. Klein, T. Bopp, C. Taube, H. Schild, E. Schmitt, et al. 2007. Mast cells are crucial for early inflammation, migration of Langerhans cells, and CTL responses following topical application of TLR7 ligand in mice. *Blood*. 110:946-953.
188. Dudeck, A., C. A. Suender, S. L. Kostka, E. von Stebut, and M. Maurer. 2011. Mast cells promote Th1 and Th17 responses by modulating dendritic cell maturation and function. *Eur. J. Immunol.* 41:1883-1893.
189. St John, A. L., C. Y. Chan, H. F. Staats, K. W. Leong, and S. N. Abraham. 2012. Synthetic mast-cell granules as adjuvants to promote and polarize immunity in lymph nodes. *Nature materials*. 11:250-257.
190. Supajatura, V., H. Ushio, A. Nakao, S. Akira, K. Okumura, C. Ra, and H. Ogawa. 2002. Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. *J Clin Invest*. 109:1351-1359.
191. Dietrich, N., M. Rohde, R. Geffers, A. Kroger, H. Hauser, S. Weiss, and N. O. Gekara. 2010. Mast cells elicit proinflammatory but not type I interferon responses upon activation of TLRs by bacteria. *Proc Natl Acad Sci U S A*. 107:8748-8753.
192. Munoz, S., R. Hernandez-Pando, S. N. Abraham, and J. A. Enciso. 2003. Mast cell activation by Mycobacterium tuberculosis: mediator release and role of CD48. *J Immunol*. 170:5590-5596.
193. Metz, M., A. M. Piliponsky, C. C. Chen, V. Lammel, M. Abrink, G. Pejler, M. Tsai, and S. J. Galli. 2006. Mast cells can enhance resistance to snake and honeybee venoms. *Science*. 313:526-530.
194. Nilsson, G., M. Johnell, C. H. Hammer, H. L. Tiffany, K. Nilsson, D. D. Metcalfe, A. Siegbahn, and P. M. Murphy. 1996. C3a and C5a are chemotaxins for human mast cells and act through distinct receptors via a pertussis toxin-sensitive signal transduction pathway. *Journal of immunology (Baltimore, Md. : 1950)*. 157:1693-1698.
195. Malaviya, R., T. Ikeda, E. Ross, and S. N. Abraham. 1996. Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha. *Nature*. 381:77-80.
196. Aoki, R., T. Kawamura, F. Goshima, Y. Ogawa, S. Nakae, A. Nakao, K. Moriishi, Y. Nishiyama, and S. Shimada. 2013. Mast cells play a key role in host defense against herpes simplex virus infection through TNF-alpha and IL-6 production. *J Invest Dermatol*.
197. Liu, A. Y., D. F. Dwyer, T. G. Jones, L. G. Bankova, S. Shen, H. R. Katz, K. F. Austen, and M. F. Gurish. 2013. Mast cells recruited to mesenteric lymph nodes during helminth infection remain hypogranular and produce IL-4 and IL-6. *Journal of immunology (Baltimore, Md. : 1950)*. 190:1758-1766.
198. Jawdat, D. M., G. Rowden, and J. S. Marshall. 2006. Mast cells have a pivotal role in TNF-independent lymph node hypertrophy and the

mobilization of Langerhans cells in response to bacterial peptidoglycan. *J. Immunol.* 177:1755-1762.

199. Vosskuhl, K., T. F. Greten, M. P. Manns, F. Korangy, and J. Wedemeyer. 2010. Lipopolysaccharide-mediated mast cell activation induces IFN-gamma secretion by NK cells. *J Immunol*. 185:119-125.
200. Kunder, C. A., A. L. St John, G. Li, K. W. Leong, B. Berwin, H. F. Staats, and S. N. Abraham. 2009. Mast cell-derived particles deliver peripheral signals to remote lymph nodes. *The Journal of experimental medicine*. 206:2455-2467.
201. Kayamuro, H., Y. Yoshioka, Y. Abe, S. Arita, K. Katayama, T. Nomura, T. Yoshikawa, R. Kubota-Koketsu, K. Ikuta, S. Okamoto, et al. 2010. Interleukin-1 family cytokines as mucosal vaccine adjuvants for induction of protective immunity against influenza virus. *J Virol*. 84:12703-12712.
202. Meng, S., Z. Liu, L. Xu, L. Li, S. Mei, L. Bao, W. Deng, L. Li, R. Lei, L. Xie, et al. 2011. Intranasal immunization with recombinant HA and mast cell activator C48/80 elicits protective immunity against 2009 pandemic H1N1 influenza in mice. *PLoS One*. 6:e19863.
203. Staats, H. F., J. R. Fielhauer, A. L. Thompson, A. A. Tripp, A. E. Sobel, M. Maddaloni, S. N. Abraham, and D. W. Pascual. 2011. Mucosal targeting of a BoNT/A subunit vaccine adjuvanted with a mast cell activator enhances induction of BoNT/A neutralizing antibodies in rabbits. *PLoS One*. 6:e16532.
204. McGowen, A. L., L. P. Hale, C. P. Shelburne, S. N. Abraham, and H. F. Staats. 2009. The mast cell activator compound 48/80 is safe and effective when used as an adjuvant for intradermal immunization with Bacillus anthracis protective antigen. *Vaccine*. 27:3544-3552.
205. Hazenbos, W. L., J. E. Gessner, F. M. Hofhuis, H. Kuipers, D. Meyer, I. A. Heijnen, R. E. Schmidt, M. Sandor, P. J. Capel, M. Daeron, et al. 1996. Impaired IgG-dependent anaphylaxis and Arthus reaction in Fc gamma RIII (CD16) deficient mice. *Immunity*. 5:181-188.
206. Galli, S. J. 1993. Mast cell deficient mice and rats with mutations of the c-kit protooncogene. *Jpn. J. Cancer Res.* 84:inside front cover.
207. Yamazaki, M., T. Tsujimura, E. Morii, K. Isozaki, H. Onoue, S. Nomura, and Y. Kitamura. 1994. C-kit gene is expressed by skin mast cells in embryos but not in puppies of Wsh/Wsh mice: age-dependent abolishment of c-kit gene expression. *Blood*. 83:3509-3516.
208. Kitamura, Y., S. Go, and K. Hatanaka. 1978. Decrease of mast cells in W/Wv mice and their increase by bone marrow transplantation. *Blood*. 52:447-452.
209. Nakano, T., T. Sonoda, C. Hayashi, A. Yamatodani, Y. Kanayama, T. Yamamura, H. Asai, T. Yonezawa, Y. Kitamura, and S. J. Galli. 1985. Fate of bone marrow-derived cultured mast cells after intracutaneous, intraperitoneal, and intravenous transfer into genetically mast cell-deficient W/Wv mice. Evidence that cultured mast cells can give rise to both connective tissue type and mucosal mast cells. *J Exp Med*. 162:1025-1043.

210. Stevens, J. and J. F. Loutit. 1982. Mast cells in spotted mutant mice (W Ph mi). *Proc R Soc Lond B Biol Sci*. 215:405-409.

211. Grimbaldeston, M. A., C. C. Chen, A. M. Piliponsky, M. Tsai, S. Y. Tam, and S. J. Galli. 2005. Mast cell-deficient W-sash c-kit mutant Kit W-sh/W-sh mice as a model for investigating mast cell biology in vivo. *Am. J. Pathol.* 167:835-848.

212. Duttlinger, R., K. Manova, G. Berrozpe, T. Y. Chu, V. DeLeon, I. Timokhina, R. S. Chaganti, A. D. Zelenetz, R. F. Bachvarova, and P. Besmer. 1995. The Wsh and Ph mutations affect the c-kit expression profile: c-kit misexpression in embryogenesis impairs melanogenesis in Wsh and Ph mutant mice. *Proc Natl Acad Sci U S A*. 92:3754-3758.

213. Galli, S. J., K. M. Zsebo, and E. N. Geissler. 1994. The kit ligand, stem cell factor. *Advances in immunology*. 55:1-96.

214. Galli, S. J., J. Kalesnikoff, M. A. Grimbaldeston, A. M. Piliponsky, C. M. Williams, and M. Tsai. 2005. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu. Rev. Immunol.* 23:749-786.

215. Nigrovic, P. A., D. H. Gray, T. Jones, J. Hallgren, F. C. Kuo, B. Chaletzky, M. Gurish, D. Mathis, C. Benoist, and D. M. Lee. 2008. Genetic inversion in mast cell-deficient (Wsh) mice interrupts corin and manifests as hematopoietic and cardiac aberrancy. *Am J Pathol*. 173:1693-1701.

216. Kawakami, T. 2009. A crucial door to the mast cell mystery knocked in. *J Immunol*. 183:6861-6862.

217. Wolters, P. J., J. Mallen-St Clair, C. C. Lewis, S. A. Villalta, P. Baluk, D. J. Erle, and G. H. Caughey. 2005. Tissue-selective mast cell reconstitution and differential lung gene expression in mast cell-deficient Kit(W-sh)/Kit(W-sh) sash mice. *Clin Exp Allergy*. 35:82-88.

218. Ebmeyer, J., U. Ebmeyer, K. Pak, H. Sudhoff, D. Broide, A. F. Ryan, and S. Wasserman. 2010. Reconstitution of the mast cell population in W/Wv mice. *Otology & neurotology : official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology*. 31:42-47.

219. Reber, L. L., T. Marichal, and S. J. Galli. 2012. New models for analyzing mast cell functions in vivo. *Trends Immunol*. 33:613-625.

220. Hohman, R. J. and S. C. Dreskin. 2001. Measuring degranulation of mast cells. *Current protocols in immunology / edited by John E. Coligan ... [et al.]*. Chapter 7:Unit 7 26.

221. Xiang, Z. and G. Nilsson. 2000. IgE receptor-mediated release of nerve growth factor by mast cells. *Clin. Exp. Allergy*. 30:1379-1386.

222. Sgonc, R. and J. Gruber. 1998. Apoptosis detection: an overview. *Experimental gerontology*. 33:525-533.

223. Martin, S., I. Pombo, P. Poncet, B. David, M. Arock, and U. Blank. 2000. Immunologic stimulation of mast cells leads to the reversible exposure of phosphatidylserine in the absence of apoptosis. *Int Arch Allergy Immunol*. 123:249-258.

224. Dvorak, A. M., R. P. Schleimer, and L. M. Lichtenstein. 1988. Human mast cells synthesize new granules during recovery from degranulation. In vitro studies with mast cells purified from human lungs. *Blood*. 71:76-85.

225. Xiang, Z., C. Moller, and G. Nilsson. 2006. Readministration of IgE is required for repeated passive cutaneous anaphylaxis in mice. *Int. Arch. Allergy Immunol.* 141:168-171.

226. Pearse, R. N., T. Kawabe, S. Bolland, R. Guinamard, T. Kurosaki, and J. V. Ravetch. 1999. SHIP recruitment attenuates Fc gamma RIIB-induced B cell apoptosis. *Immunity*. 10:753-760.

227. Xiang, Z., A. J. Cutler, R. J. Brownlie, K. Fairfax, K. E. Lawlor, E. Severinson, E. U. Walker, R. A. Manz, D. M. Tarlinton, and K. G. Smith. 2007. FcgammaRIIb controls bone marrow plasma cell persistence and apoptosis. *Nat. Immunol.* 8:419-429.

228. Tzeng, S. J., S. Bolland, K. Inabe, T. Kurosaki, and S. K. Pierce. 2005. The B cell inhibitory Fc receptor triggers apoptosis by a novel c-Abl family kinase-dependent pathway. *J. Biol. Chem.* 280:35247-35254.

229. Hjelm, F., F. Carlsson, A. Getahun, and B. Heyman. 2006. Antibody-mediated regulation of the immune response. *Scand J Immunol*. 64:177-184.

230. Getahun, A. and B. Heyman. 2006. How antibodies act as natural adjuvants. *Immunol. Lett.* 104:38-45.

231. Getahun, A., J. Dahlstrom, S. Wernersson, and B. Heyman. 2004. IgG2a-mediated enhancement of antibody and T cell responses and its relation to inhibitory and activating Fc gamma receptors. *Journal of immunology (Baltimore, Md. : 1950)*. 172:5269-5276.

232. Heyman, B., L. Pilstrom, and M. J. Shulman. 1988. Complement activation is required for IgM-mediated enhancement of the antibody response. *The Journal of experimental medicine*. 167:1999-2004.

233. Diaz de Stahl, T., J. Dahlstrom, M. C. Carroll, and B. Heyman. 2003. A role for complement in feedback enhancement of antibody responses by IgG3. *The Journal of experimental medicine*. 197:1183-1190.

234. Schwartz, L. B. 1985. Monoclonal antibodies against human mast cell tryptase demonstrate shared antigenic sites on subunits of tryptase and selective localization of the enzyme to mast cells. *Journal of immunology (Baltimore, Md. : 1950)*. 134:526-531.