A shot in the arm for mast cells

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The search is on for for vaccine adjuvants that boost the innate immune response and complement existing adjuvants. Mast cell activators may be one option (pages 536–541).

In the two centuries that have elapsed since Jenner's first recorded vaccine trial in history, immunologists have proposed using a variety of adjuvants, substances that boost the immunogenicity of vaccines. But only alum, an aluminum salt, is globally licensed for use[1]. Alum, however, does not induce strong cellular immunity, and there is an urgent need for new adjuvants that induce different types of immune responses that might combat a variety of pathogens.

In this issue of *Nature Medicine*, McLachlan *et al.*[2] introduce a new potential cellular target for adjuvants—the versatile mast cell, perhaps best known for its role in allergic reactions. The researchers find that molecules that activate mast cells stimulate protective immune responses against infections, emphasizing that these cells help orchestrate the adaptive immune response[2].

The findings are part of a trend in adjuvant research examining the value of factors that stimulate the innate immune response. Several new targets have emerged from this research, including Toll-like receptors (TLRs), as well as other receptors (non-TLRs)[3–5]. TLRs and non-TLRs sense viral or microbial stimuli and activate dendritic cells (DCs) to stimulate adaptive immune responses[4]. DCs have also emerged as preeminent targets for vaccine adjuvants—not surprising, given their central role in sensing microbial or adjuvant stimuli and translating this information to modulate the strength, quality and duration of adaptive immune responses[6].

Mast cells are hematopoietically derived cells that are scattered throughout the body, particularly at sites of pathogen entry such as the skin and many mucosal tissues[7,8]. They are key effector cells in allergic reactions involving T helper type 2 (T_H2)- and IgE-associated immune responses. Thus, in sensitized hosts, antigen-IgE complexes activate mast cells, resulting in the release of an array of inflammatory mediators such as histamine, leukotrienes, prostaglandins and tumor necrosis factor-α (TNF-α)[7,8]. There is a growing appreciation of the diverse role of these cells in host defense and perhaps autoimmunity—including innate sensing of microbes and viruses through TLRs, recruitment of neutrophils, DCs and T lymphocytes to the sites of inflammation, and even direct antigen presentation to T cells in the context of major histocompatibility complex class I and II molecules[7,8].

Mast cells are activated by numerous factors. In addition to classic antigen-driven IgE-dependent stimuli (antigen complexed to IgE), these factors include a structurally diverse collection of cationic peptides and cationic polymers that stimulate the release of mast cell granule constituents, such as histamine and TNF-α (refs. 7,8). Such compounds, known as 'secretagogues', are thought to stimulate mast cell activation by activating G_i proteins either indirectly through cell surface receptors or directly by crossing the plasma membrane[9].

The authors evaluated several well-known mast cell activators, including compound 48/80 (c48/80), MC degranulating peptide (MCD)

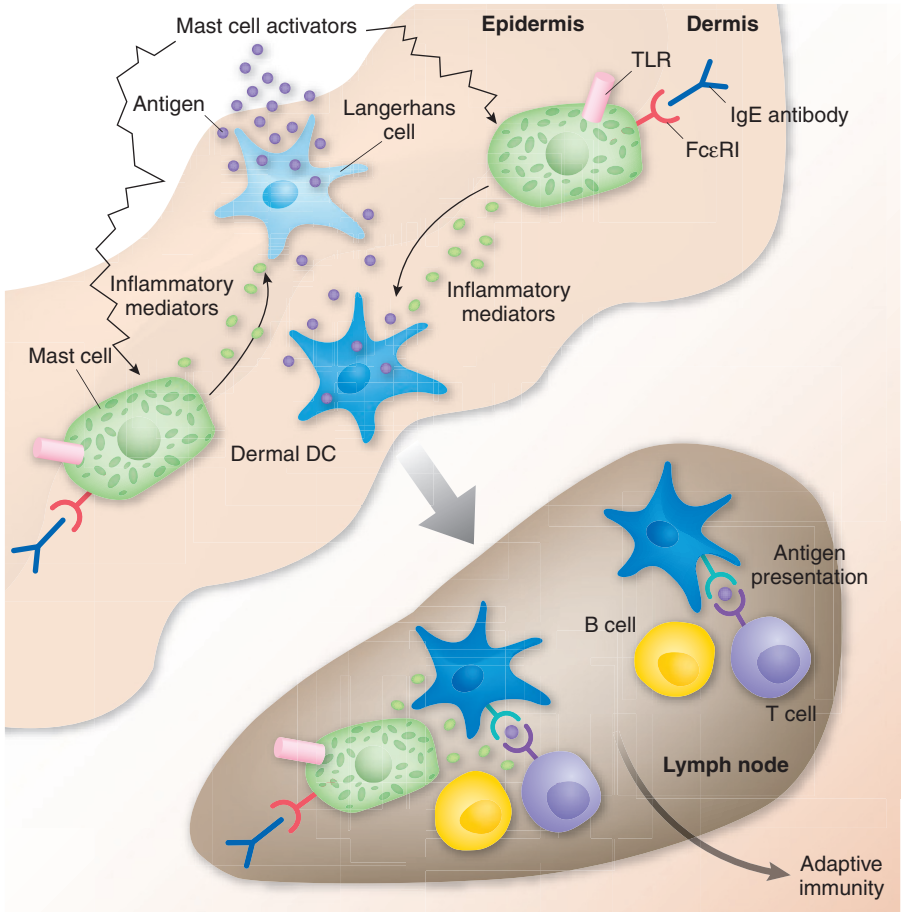

Figure 1 How mast cell adjuvants might work. As McLachlan *et al.*[2] report, injections of mast cell activators, such as c48/80, plus antigen may result in recruitment and activation of mast cells in the skin. Release of inflammatory mediators by the mast cells could lead to activation of the Langerhans cells and dermal DCs containing the injected antigen and to their migration to the draining lymph node. In the lymph node, the DCs will present antigens to T helper cells, resulting in T cell–B cell collaboration and formation of antigen-specific antibody responses. It is also possible that mast cells in the periphery may migrate to the lymph nodes and contribute to the induction of T and B cell responses there, either through antigen presentation or through release of inflammatory mediators. FcεRI, Fcε receptor I.

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and catestatin ($CgA_{344–364}$), for their ability to initiate an immune response[2].

The researchers immunized wild-type mice with c48/80 mixed with a low dose of antigen—protective antigen (PA) from *Bacillus anthracis*[2]. This procedure resulted in a robust increase in IgG antibodies specific for PA 7 days after immunization. A similar immunization of mice deficient in mast cells resulted in a greatly diminished response—a PA-specific IgG response ten times lower than in wild-type mice. Notably, this deficit was rescued by reconstituting the mice with mast cells at the site of injection.

The response to all three mast cell activators seemed to operate through the activation of DCs, as shown by experiments in mice with DCs transiently deleted at the time of immunization. Similar experiments in mice deficient in TLR-mediated signaling showed that TLRs did not seem to be involved in the response. The response, however, did require mast-cell derived TNF, which is stored in granules and which mobilizes lymphocytes and DCs[10].

Furthermore, the authors found that c48/80 or $CgA_{344–364}$ could stimulate a mucosal response in the saliva, vagina and gut[2]. In fact, the response was comparable in magnitude and duration (up to 6 months) to that stimulated by cholera toxin, considered a gold standard for mucosal adjuvants.

Finally, the authors demonstrated the protective capacity of the antibody response in preventing anthrax toxin–mediated cell death of macrophages *in vitro*[2]. In addition, to determine the protective capacity of the antibody response *in vivo*, the authors employed a vaccinia virus challenge model, using an envelope component of the vaccinia as an immunogen, and showed some degree of protection[2].

These studies demonstrate an unappreciated role for mast cell activators in stimulating robust antibody responses via a mechanism involving both mast cells and DCs (**Fig. 1**).

Future studies could buttress these findings by addressing the underlying mechanisms and by further exploring the relevance for vaccinology. Can the immunogenic effects of these compounds be attributed solely to mast cells? Probably not, as there was still robust—though tenfold reduced—induction of immune responses in mice deficient in mast cells. The mast cell–deficient and wild-type mice may also have intrinsic differences in the strength of their immune response, which will need to be addressed with other strains of mast cell–deficient mice such as $Kit^{W\text{-}sh}$ mice.

The role of DCs in mediating the immunogenicity of the mast cell activators will need further exploration. The experiment using the conditional deletion of DCs seems compelling, although there is no preferential increase in the numbers of DC subsets in the lymph nodes, nor is it clear to what extent the DCs are activated. Indeed, whether c48/80 exerts a direct effect on DCs, or whether the observed effects on DCs are due to some bystander activation, has not been addressed. Furthermore, precisely how, where and when DCs and mast cells cooperate to generate immunity is unknown (**Fig. 1**).

Vaccinologists will be interested in whether these compounds offer unique advantages, either scientific or economic, over emerging adjuvants such as TLR ligands or already licensed adjuvants—such as alum, or the TLR4 ligand monophosphoryl lipid A, recently licensed in Europe. Further comparative studies, as well as a rigorous evaluation of the toxicity profile of mast cell–dependent adjuvants, are necessary before the use of these adjuvants in vaccines can be entertained.

These issues notwithstanding, the paper by McLachlan *et al.*[2] highlights an important role for mast cells in regulating the humoral immune response and offers a tantalizing glimpse into their potential as cellular targets of vaccine adjuvants.

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