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Use and limitations of alum-based models of allergy

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What do we know about the immunopathology of allergic disease and how did we learn it?

Early observations in patients with allergic diseases such as asthma, eczema and food hypersensitivities suggested that a deregulated immune response to environmental stimulants resulted in the pathology associated with atopic disease. Animal models of allergic disease were established in the early 1990s primarily utilizing the mouse, rat or guinea-pig to mimic the major features of atopic disease, which allowed for the study of early sensitization events and dissection of the responsible pathways resulting in end-organ pathology. Despite the differences between mouse and human biology [1], the use of genetically modified mice in particular, has led to a tremendous understanding of the T helper type-2 (Th2) nature of allergic disease as well as the various signalling components involved in generating and propagating Th2-mediated inflammatory disorders (for review see reference [2]). Understanding the immunopathogenesis of atopic disease has in turn facilitated a focused search for candidate susceptibility genes. Our understanding of the genetics of asthma has seen a tremendous advance in the past decade and has provided us with valuable insight into the risk and development of disease [3], but has also raised further questions that can be transferred back to the bench (or cage) for clarification.

Different models provide insight from different vantage points. The majority of allergic airway and some of the food hyper-sensitivity models use a potent pro-Th2 adjuvant called aluminium hydroxide (commonly referred to as alum). Kung et al. first developed a Th2-based model of allergic disease using alum to dissect the role of particular cells, cytokines and signalling pathways in asthma [4]. Alum-based models of allergic airway disease typically use a dose of 10–100 mg of protein antigen adsorbed (see ‘Caveats of alum-based models’) onto 1–2 mg of aluminium hydroxide administered intraperitoneally or subcutaneously. The immune response is then boosted approximately 10–14 days later with a repeated injection. Around this time-point, there is evidence of a systemic type II immune response with increased IgG1 and IgE antibody titres along with primed and differentiated helper Th2 cells producing IL-4, IL-5 and IL-13 in the mediastinal draining lymph nodes (but interestingly, not the spleen following intraperitoneal immunizations) [5]. Airway challenge activates these Th2 cells and draws a potent eosinophil-dominated inflammatory

response to the lung and a further systemic boosting of the antibody response (Fig. 1). The simple readouts of these models are markers of allergic disease: pulmonary eosinophilia, lymph node T cell responses and serum IgE and IgG1. Airway hyper-reactivity is a critical parameter used to diagnose asthma in humans, yet the subjectivity of the methods available in mice limits its usefulness and is therefore one major shortcoming of these small animal models of asthma.

While this alum-based model has limitations, it also presents some useful advantages. There is no doubt that alum effectively activates an immune response to otherwise non-immunogenic protein antigens and, in addition, induces strong Th2 polarization regardless of murine strain background. By quickly generating a reproducible priming step with alum, it is possible to study in depth the challenge phase of disease, which is typically the phase we are dealing with in allergic patients. Mechanism of lung injury, methods of interfering with ongoing inflammation and lymphocyte effector responses can all be studied. In addition, this model has been applied to both acute and chronic models of airway disease, depending on the duration of airway exposure [6].

Using this model of allergic airway disease, Bortolatto et al. in the current issue of *Clinical Experimental Allergy* examine the effect of lipopolysaccharide (LPS) administered subcutaneously with alum during Th2 priming [7]. The addition of LPS inhibited Th2 responses in a dose-dependent manner without inducing a Th1-associated inflammatory response. This inhibition was dependent on both TLR4, the receptor for LPS, and MyD88, an intracellular adaptor protein critical in most TLR signalling pathways. This work raises a new, interesting extension of the old Th1 vs. Th2 principle of mutual antagonism of cell differentiation with concomitant use of Th1-favouring (i.e. LPS) and Th2-favouring (i.e. alum) adjuvants preventing complete effector development altogether. Of course, as with any conclusion inferred from an animal model, one must ask how this finding applies to human allergic disease.

Caveats of alum-based models

A model is only a model and as such will not correlate with certain aspects of human disease. They can be used to dissect the interaction of specific gene defects with specific environmental cues, but the full complexity of human disease cannot be modelled. One very clear non-physiologic aspect of the alum-based models is the site of sensitization. The most relevant route of sensitization for asthma models of human disease is through the airways or skin, yet these models are difficult, slow and labour intensive. While effective and reproducible, intraperitoneal sensitization in the alum-based models initiates immunity using a population of antigen presenting cells (APCs) that are resident in a unique environment and may have a different baseline activation status and effector function than the APCs most likely involved in sensitization in human disease, the pulmonary dendritic cells or Langerhans cells [8]. Therefore, comparing priming effects by intranasal, oral or cutaneous routes to alum-based intraperitoneal models may lead to erroneous conclusions.

In addition, alum-based models have proved ineffective at evaluating interventions aimed at aborting the development of allergic disease. This should not be surprising given the

divergent mechanisms likely at work in alum-induced vs. airway or cutaneous sensitization. Testing the effect of interventions during alum administration assumes that alum is simply a carrier of antigen without inherent immunostimulatory properties. Yet obviously, alum would not be an adjuvant if it did not have some inherent ability to activate the immune system. While alum results in effective Th2 responses in animal models, it does not likely mirror the sensitization process to allergens (i.e. the initial immunologic priming events) and, therefore, this adjuvant does not provide an ideal model to study the mechanism of Th2 allergen sensitization. For example, evaluating the role of a susceptibility gene in the genesis of atopic disease is probably inappropriate in an alum-based model because it would ignore the gene–environment interactions that are presumably required to develop allergy. As alum induces a strong Th2 response that can be conveniently called to the lung in asthma models, this model is instead ideal to evaluate the factors involved in Th2-mediated inflammation in the lung during *challenge* periods (Fig. 1). But until we fully understand how alum regulates naïve T cell activation and Th2 differentiation, caution must be exerted in making conclusions from interventions administered during the priming phase. Other animal models of allergic disease exist using different adjuvants or antigens that themselves have immunostimulatory properties such as proteases, Toll-like receptor (TLR) agonists or other stimulators of the innate immune system [9, 10]; these models may more accurately reflect the sensitization steps in atopic patients and are therefore better suited for study of interventions during the sensitization phase.

While aluminium hydroxide has been a convenient Th2 adjuvant in these allergy models, it is useful to take a step back and evaluate what we know about the mechanism of action of this key vaccine component [11]. In fact, we know quite a lot about how it physically interacts with antigens and with tissues [12]. ‘Alum’ typically refers to a class of chemical compounds that form insoluble gel-like precipitates; antigens are adsorbed onto alum primarily via electrostatic interactions [13]. Adsorption of antigen with the consequential slow release of antigen following immunization is thought to be one mechanism by which alum acts as an adjuvant. Beyond this physical ‘depot’ effect, how does alum regulate naïve lymphocyte activation and Th2 differentiation?

Using genetically modified mice, we know that the adjuvant activity of alum is independent of IL-4, the IL-4 receptor, Stat6, IL-5, IL-6 and TNF- α . Whether alum directly induces dendritic cell activation is still controversial [13]. Many effective adjuvants induce immunity by stimulating components of the innate immune system; however, there does not seem to be a role for TLRs in the mechanism of action of alum. Piggott et al. [8] and Schnare et al. [14] showed that Th2 immune responses to protein antigens in alum are intact in MyD88-deficient mice. Gavin et al. further evaluated the effect of alum on B cell responses in MyD88 and TRIF double-deficient mice (mice deficient in all known TLR signalling) and found no defect in antibody production; however, these studies used haptenated antigens, which confounds the interpretation of what is controlling immunity in this model [15]. We have recently found that aluminium adjuvants activate a different class of pattern recognition receptors (PRR), the intracellular NOD-like receptors (NLR) [16]. These PRRs are sensitive to markers of cellular stress (such as uric acid crystals and ATP) and induce secretion of potent pro-inflammatory cytokines such as IL-1 β , IL-18 and IL-33 (for review see reference [17]). *In vitro*, TLRs and NLRs synergize to induce IL-1 β production, although it is not

known if this same situation applies *in vivo*. How this pathway (or a different one) directs Th2 differentiation still remains a mystery; however, it is likely that alum is an effective adjuvant because it uses multiple pathways to activate the immune system.

Given this unique priming phase with aluminium adjuvants, what can we conclude from studies that introduce immune modulators during immunization? The work by Bortolatto et al. argues that simultaneous TLR (LPS) and NLR (alum) stimulation *in vivo* is mutually inhibitory (the pro-Th1 effects of LPS are dampened by alum and the pro-Th2 effect of alum is inhibited by LPS). It is intriguing to speculate that the activation of two different classes of PRRs provides conflicting differentiation signals and this accounts for the absence of either response. However, one cannot infer from these findings that giving LPS to humans during initial allergen exposure will inhibit Th2 responses, as we do not know whether the stimulus initiating Th2-mediated disease in atopic patients is the same as an alum-derived stimulation. The authors do not claim this to be the case and instead make an interesting connection to alum-based desensitization methods employed by allergists to modify ongoing Th2 inflammation (see 'The human translation of alum-based models').

The human translation of alum-based models

While conclusions from these alum-based models should not be used to determine how patients develop allergen-specific Th2 responses, they have interesting applications to therapeutic interventions. Indeed, antigens in aluminium hydroxide have been used in allergen immunotherapy. One would have been unlikely to predict this outcome based on murine models, which show dramatically enhanced, alum-dependent, Th2 sensitization to almost any chosen allergen. In contrast, subcutaneous injections of escalating doses of allergen in alum over prolonged periods reduces allergen-specific IgE in atopic patients and enhances 'neutralizing' antibodies (including IgG4) [18]. The mechanism underlying this observation remains unknown but it has been proposed to result from a Th2 to a Th1 or regulatory T cell shift. The work by Bortolatto et al. suggests that it is possible to abrogate the T cell response to an allergen in alum without deviation to another T cell profile by co-administering opposing immunostimulatory signals. Although they did not formally test if this method would work in previously sensitized animals, these findings might be translated into novel immunomodulatory therapies for allergic disease.

Until we can accurately predict those individuals who have a significantly increased risk for the development of allergic disease, we cannot intervene during the primary immune response. Instead, we are faced with patients who are not only sensitized but have been repeatedly challenged with the offending antigen. Intervening at this stage is infinitely more difficult and requires re-education of the immune system (something we fail at in general in immune-mediated diseases). Models that initiate Th2 immunity that can then be modulated during on-going inflammation are critical to the design of new interventions in allergic disease. Alum-based models provide a rapid, easy and reproducible system for such studies.

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Fig. 1.

Stages of alum-based murine asthma models. Mice are typically primed/sensitized (i.e. naïve T cell activation) to a protein antigen (often ovalbumin) in combination with alum (aluminum hydroxide gel) on days 0 and 10. Most models use either intraperitoneal (IP) or subcutaneous (SC) injections. During this time, antigen presenting cells (APCs) receive activation signals, presumably from the adjuvant, to endocytose antigen, process it, migrate to the draining lymph nodes and present peptides to circulating naïve T cells. It is also in the secondary lymphoid organs where cognate T : B cell interactions result in B cell activation with subsequent affinity maturation and isotype switching to produce antigen-specific IgE and IgG1. Intranasal (IN) challenge with antigen recruits primed lymphocytes to the lung and initiates a fulminant eosinophil-dominated inflammatory response. It is during this second phase that this alum-based model is most useful for the study of intervention in allergic disease.