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Previews

Immunity by AS03ation: The natural adjuvantage

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Humans do not respond equally to vaccination. To investigate why, Mulè et al. developed a multimodal framework and found that high responders after unadjuvanted influenza vaccination exist in a naturally adjuvanted state, mimicking innate immunophenotypes following AS03-adjuvanted vaccination. This highlights biological factors that set apart high-antibody responders and how adjuvants can boost innate immune cues to improve humoral immunity.

Vaccination is considered one of the most successful medical interventions in history and has saved millions of lives. However, vaccines are not equally effective for everyone, with some individuals generating robust antibody responses while others fail to seroconvert. This raises the question of which biological factors make someone a high-antibody responder and others low-antibody responders. Some intrinsic factors, such as age and sex, may immediately come to mind; however, it has become apparent that these factors cannot account for all the variability that is observed.[1] In recent years, the technical advancements of single-cell sequencing modalities have allowed for high-throughput profiling of transcriptomics, surface protein expression (CITE-seq), and chromatin accessibility (ATAC-seq) that have allowed for systematic immunological assessments of immune responses. Despite the amount of data that we are now able to generate at the single-cell level, it remains a challenge to interpret the data into actual biological insights. This interpretation is further complicated by substantial interpersonal variation of the immune system and our limited understanding of how baseline immune states shape and potentiate the immune response quality and quantity. In this manuscript by Mulè et al.,[2] the authors generated a multimodal dataset of human peripheral blood mononuclear cells (PBMCs) and integrated human population, temporal, and single-cell variation to identify biological factors that separate high- from low-antibody responders (Figure 1).

The study participants were vaccinated with the standard-of-care unadjuvanted trivalent seasonal influenza vaccine in combination with a monovalent pandemic H1N1 2009 strain. Within one day of vaccination, the major innate immune cues were the activation and sensing of type I interferons by $CD14^+$ and $CD16^+$ monocytes. Numerous signaling pathways associated with type I interferon were induced, including interferon-induced transcription factors (e.g., STAT1, IRF7), increases in adhesion molecules and antigen-presentation molecules, and expression of interferon-stimulated genes (ISGs). These pathways together place monocytes in an anti-viral state as well as position them as antigen-presenting cells, which can bridge innate and adaptive immunity and lead to robust induction of humoral responses. Strikingly, the authors identified that these signatures and the induction of gene sets associated with phagocytosis and cytoskeletal rearrangement were increased in high responders relative to the low responders. These data suggest that high responders naturally have a higher set-point for inducing innate immune responses when compared to individuals who fail to induce robust antibody responses. Recent studies have similarly found that innate immune responses in monocytes at baseline are predictive of an individual mounting an antibody response following vaccination[3] and protection from influenza virus infection.[4] Collectively, these studies show that some people are in a heightened innate immune state, or "naturally adjuvanted," and thus more likely to be high-antibody responders following vaccination.

While naturally adjuvanted individuals are primed to be high responders, low responders remain unable to mount significant innate immune responses. This raises the question, how can vaccine strategies convert low responders into high responders? Adjuvants are frequently used to increase the immunogenicity of vaccines and to overcome potential negative impacts posed by self-intrinsic factors. AS03 is an oil-in-water emulsion adjuvant that has been used in numerous vaccine formulations with great success, including influenza virus vaccines. Despite this, the underlying immunological mechanisms contributing to the increased immunogenicity are not fully understood. To determine the impacts of AS03 on these early innate immune cues, the authors tracked innate immune responses in individuals who received an AS03-adjuvanted H5N1 vaccine relative to individuals who received the unadjuvanted seasonal influenza vaccine. PBMCs were collected at baseline, day 1, and day 7 from both groups and used for transcriptomic analysis, which allowed them to dissect the immunophenotypes associated with AS03-adjuvanted and unadjuvanted influenza vaccines. Within 24 h, the AS03 vaccine induced innate immune responses, specifically monocytes with a type I interferon-induced inflammatory phenotype. Other studies analyzing innate immune responses following AS03-adjuvanted H5N1 vaccination or phase I clinical trial with a chimeric hemagglutinin revealed similar patterns in monocyte populations, including induction of type I interferon responses and increased antigen presentation.[5,6] Both studies were linked to improved humoral immunity relative to unadjuvanted vaccination, further

emphasizing the critical role of AS03-primed innate immune responses in orchestrating and potentiating humoral immunity.

AS03 has been shown to increase antibody titers and breadth to protect against influenza clades beyond those included in the vaccine.[7] Mulè and colleagues show that within one day of immunization, naive B cells from individuals who received the AS03-adjuvanted vaccine downregulate pro-apoptotic genes while increasing co-stimulatory molecules, including CD40.[2] Moreover, antibody avidity was increased, indicating that the adjuvant improves antibody magnitude, breadth, and quality. The authors propose that AS03 increases the clonal diversity of naive B cells recruited into the germinal center, contributing to increased binding breadth of serum antibodies. A recent study using an AS03-adjuvanted COVID-19 vaccine revealed that the adjuvant improved not only the magnitude, binding breadth, and durability of circulating antibodies but also increased the frequency of antigen-specific memory CD4$^+$ T cells and memory B cells.[8] Mechanistically, AS03 is known to induce danger-associated molecular patterns (DAMPs) that create endoplasmic reticular stress and upregulation of pro-inflammatory cytokines in myeloid cells, which increases the T follicular helper cell response and antibody avidity.[9] Therefore, AS03 functions to provide early innate cues to increase monocyte activation and orchestrate effective adaptive immune responses.

AS03-adjuvanted vaccines may improve vaccine immunogenicity and effectiveness by converting low-antibody responders to high-antibody responders through boosting of innate immune responses, particularly myeloid cell activation and function, to better orchestrate adaptive immune responses. The innate immune cues provided by AS03 are likely the reason that the seroconversion rate for AS03-adjuvanted vaccines is nearly 100%.[5,6] Moreover, AS03 improves B cell activation and co-stimulation, which could lead to the observation of improved antibody diversity and durability or slower decay rate in total antibody titers.[8] While AS03 induces superior antibody responses relative to unadjuvanted vaccines, it is not known whether naturally adjuvanted individuals who receive an unadjuvanted vaccine also have improved antibody breadth and durable humoral immunity. Therefore, further research on naturally adjuvanted individuals may provide insights that can be further harnessed to improve vaccine design and inform the inclusion of adjuvants.

A major finding of this study is that the innate immune cues turned on by an AS03-adjuvated vaccine mimic the innate immune setpoint found in naturally adjuvanted individuals at baseline. However, what determines if a person is naturally adjuvanted? To address this, the authors took baseline PBMCs from vaccinees who went on to be high-antibody responders versus low-antibody responders and found that CD14$^+$ monocytes from high responders more potently respond to lipopolysaccharides (LPS). These data suggest that pathogen-associated molecular patterns (PAMPs) pre-prime monocytes in high responders to be more active before and after vaccination. While Mulè and colleagues only studied the response to LPS treatment, Fourati et al. revealed that both bacterial and viral PAMPs mimicked naturally adjuvanted set points in people who go on to become high-antibody responders following vaccination.[3] This highlights the role of viral and bacterial PAMPs in creating a naturally adjuvanted state. AS03 induces DAMPs, which can engage similar innate immune pathways as PAMPS,[9] which may provide insight into the similarities between immune responses in naturally adjuvanted individuals and people who receive an AS03-adjuvanted vaccine. However, it remains unknown whether high responders have inherently higher levels of PAMPs that place monocytes in a pre-primed state or whether these individuals have polymorphisms in immune sensing pathways that could lead to a higher sensitivity to PAMPS and a higher innate immune setpoint. Moreover, antibiotics treatment reduces humoral immunity following unadjuvanted influenza vaccination in humans and is linked to reduced inflammasome activation,[10] suggesting a critical role of bacterial-associated PAMPs in regulating antibody responses following vaccination. While numerous studies have highlighted a role of the microbiome in improving vaccine efficacy, it remains unclear how the microbiome and immune system synergize to improve vaccine-elicited immune responses. Moreover, the role of distinct molecular PAMPs deriving from specific commensals and pathogens in regulating the naturally adjuvanted state remains unclear.

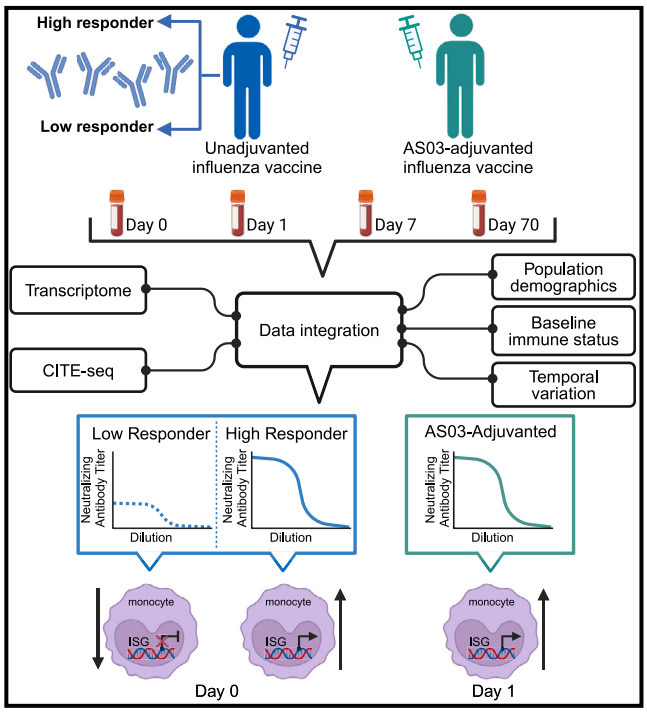

Figure 1. Multimodal single-cell sequencing reveals a naturally adjuvanted innate immune setpoint predictive of seroconversion

Multimodal single-cell sequencing methods integrating human population, temporal, and single-cell variation identify biological factors that separate high- from low-antibody responders after unadjuvanted influenza vaccination. High-antibody responders have innate transcriptional signatures enriched for interferon-induced genes (ISGs) in monocytes at baseline, mimicking innate immunophenotypes observed early after AS03-adjuvanted vaccination. Figure made using Biorender.

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By generating a framework to analyze large multimodal datasets, Mulè et al. have helped define the landscape of baseline immune phenotypes and identify whether someone becomes a high- or low-antibody responder after vaccination.[2] Linking baseline immune setpoints to early vaccine responses has implications for vaccine design and adjuvant development. This approach has the potential to be incredibly powerful for identifying correlates of vaccine immunogenicity as well as use in correlates of protection and susceptibility studies.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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Non-canonical IKKs side with N4BP1 against the family

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The ubiquitin-binding endoribonuclease N4BP1 is a critical immunosuppressor, but the mechanism by which it acts to constrain TLR-induced inflammatory cytokine production has remained unclear. In this issue of *Immunity*, Gitlin et al. find that N4BP1 works in concert with the non-canonical IκB kinase (IKK) to limit activity of the IKK complex.

Toll-like receptors (TLRs) comprise a family of pattern recognition receptors integral to innate immunity. TLRs are able to sense both foreign and "self" molecules that pose a threat to the host, initiating a rapid inflammatory response aimed at restoring immune homeostasis. Upon activation, TLRs trigger intracellular signaling cascades via the adaptor molecules MyD88 and/or Toll-interleukin-1 receptor domain-containing adaptor protein inducing interferon beta (TRIF) that drive transcriptional responses for the production of inflammatory mediators.[1] While signaling through MyD88