Learning vaccinology from viral infections

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This issue of the *Journal of Experimental Medicine* celebrates and honors the life of Ralph Steinman (1943–2011), winner of the 2011 Nobel Prize in Physiology or Medicine. Ralph's science was rooted in fundamental discovery with the goal of translating these findings into clinical medicine. He recognized the power of immunology in treating human disease and passionately championed studies on vaccine design, immune therapy, and human immunology. One particular collaborative effort between the Steinman and Sekaly laboratories resulted in a paper published in this issue of the journal.

As a result of selective pressure exerted by pathogens, both innate and adaptive human immune responses have evolved to protect us from the potentially fatal consequences of infectious diseases (Coffman et al., 2010; Sallusto et al., 2010). Pattern recognition receptors (PRRs) sense microorganisms to alert the host to the invading pathogen (Coffman et al., 2010) and induce immediate nonspecific immune responses that can partially curtail the growth and dissemination of the microbe. More importantly, these innate responses also shape the magnitude, quality, and durability of adaptive immune responses (Pulendran and Ahmed, 2006). It is the adaptive T and B cell response that eliminates the infection and confers long-term protective immunity against the same pathogen. The durability of protective immunity after an acute infection can be quite remarkable and, in some instances, can last for a lifetime (Ahmed and Gray, 1996). It was this observation that led to the use of attenuated live microbes for vaccination, which resulted in the development of our most successful vaccines against diseases such as smallpox, polio, mumps, rubella, measles, and yellow fever (Plotkin et al., 2008).

The live attenuated yellow fever virus vaccine (YFV-17D) was developed in

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the late 1930s by Max Theiler at The Rockefeller Foundation by serial passage of a virulent YFV strain in cell cultures prepared from embryonated chicken eggs (Theiler and Smith, 1937). This clever trick of passaging a virulent human virus in a different host cell in vitro to alter the virus properties resulted in the selection of a YFV strain (17D) that was attenuated but still retained immunogenicity. Within a few years of its introduction, the live YFV-17D vaccine dramatically reduced the incidence of the disease in humans, and to this day it remains one of the most successful vaccines ever developed. Max Theiler was awarded the 1951 Nobel Prize in Physiology or Medicine for his findings on vaccination against yellow fever.

The YFV-17D strain replicates in humans to sufficient levels to induce potent T and B cell responses, but the infection is rapidly cleared with minimal or no clinical symptoms. In many ways this live viral vaccine captures the right balance between attenuation and immunogenicity and confers protective immunity that can last for decades or longer (Monath et al., 2008). Protection against YFV is mediated primarily by circulating antibody, and a single YFV-17D immunization can generate neutralizing antibody responses that persist for up to 30 yr. This vaccine also induces highly polyfunctional and long-lived CD4 and CD8 T cell responses (Gaucher et al., 2008; Miller et al., 2008; Akondy et al., 2009). Thus, the YFV-17D vaccine offers an opportunity to understand how an acute viral

infection generates both humoral and cellular long-term immunity in humans and to apply this knowledge to developing new vaccines.

It is of particular interest to define the innate responses that are necessary for generating such effective T and B cell immunity. This information will provide a framework for the rational development of adjuvants to enhance vaccine efficacy. Accordingly, recent work presented detailed longitudinal analysis of adaptive immune responses induced by the YFV-17D vaccine in humans, along with systems biology data documenting innate gene expression signatures associated with these adaptive responses (Gaucher et al., 2008; Querec et al., 2009). These studies revealed that YFV-17D triggers multiple Toll-like receptors (TLRs), as well as RIG-I and MDA-5, and identified innate gene signatures that predict immunogenicity of the vaccine (Querec et al., 2006, 2009; Gaucher et al., 2008). These systems biological approaches offer unprecedented opportunities to study the human immune response and to describe the component participants (genes, miRNAs, proteins, and cells) of an immune response to a vaccine or pathogen (Germain, 2010; Pulendran et al., 2010). The ultimate goals of this approach, as applied to vaccinology, are to delineate novel molecular pathways by which vaccines mediate protective immune responses and to identify signatures that will predict vaccine immunogenicity and efficacy.

In this issue, the paper from the laboratories of Steinman and Sekaly (Caskey et al., 2011) uses a systems biology approach to ask whether an adjuvant can mimic the innate signature

of the YFV-17D vaccine. The authors examined the innate immune response to the synthetic double-stranded RNA (poly ICLC), a ligand for TLR3 and MDA-5, in 12 healthy adult volunteers. Eight subjects were injected subcutaneously with 1.6 mg of clinical grade poly ICLC (a dose used previously in phase I trials). The remaining four subjects represented the placebo group and received sterile saline. After poly ICLC administration, subjects developed erythema and induration at the site of injection and showed mild transient flu-like symptoms, but no serious adverse events were reported and there were no significant changes in total blood cell counts or serum chemistry. Thus, in this small cohort, poly ICLC appeared to be safe with tolerable side effects. They next assessed the molecular signatures induced by poly ICLC using transcriptional profiling. Whole blood samples were collected just before poly ICLC or placebo administration and at multiple time points thereafter (6 and 12 h and 1, 2, 3, 7, 14, and 28 d). Bioinformatics analysis revealed similar gene expression responses to poly ICLC among the volunteers, with a peak response at day 1 for five out of eight individuals and at 12 h for the remaining three. In all subjects, responses returned to baseline by day 7. More than 200 genes were differentially expressed in the adjuvant group relative to the placebo group. Notably, there was no differential expression of genes induced in the placebo group. Poly ICLC induced expression of genes encoding TLR7 and TLR4, the RIG-I and MDA-5 viral sensors, and transcription factors involved in type I IFN responses (IRF7, IRF5, and IRF1). Many affected pathways involved innate immunity, including IFN signaling, NF-κB signaling, DC maturation, antigen presentation, and inflammasome signaling.

The authors then compared the gene expression profiles induced by poly ICLC with those induced by the live YFV-17D vaccine. Pathway analysis revealed up-regulation of many similar transcriptional and signal transduction pathways at day 1 after poly ICLC administration and day 7 after the live YFV-17D vaccine. Of note, poly ICLC induced

the expression of TNFSF13B (BAFF), which binds TNFRSF17 (BCMA), a protein which regulates differentiation of antibody-producing cells. Importantly, *TNFRSF17* is part of the gene signatures that predict the magnitude of the antibody responses to the yellow fever vaccine (Querec et al., 2009) and the inactivated seasonal influenza vaccine (Nakaya et al., 2011). Poly ICLC also increased the expression of genes encoding components of the complement pathway (C1QB, C3AR1, and SERPING1), as well as of EIF2AK2, which encodes protein kinase RNA-activated (PKR), a viral sensor that mediates the integrated stress response (García et al., 2006). Interestingly, the expression of genes encoding C1QB and EIF2AK4 (a homologue of EIF2AK2) have been shown to be predictors of the CD8 T cell response to the YFV-17D vaccine (Querec et al., 2009). Thus, there appears to be a striking similarity in the innate signatures induced by the adjuvant poly ICLC and the viral vaccine YFV-17D.

The well designed human study by Caskey et al. (2011) represents an important first step in identifying adjuvants that mimic the innate signatures of a successful viral vaccine. However, it is worth noting that viral infections are likely to "push many more buttons" given the broader range of interactions they have with the immune system. For example, YFV-17D stimulates multiple TLRs and is likely to target a wider spectrum of cell types, including several DC subsets that express these receptors. Furthermore, previous studies have shown that distinct but overlapping signatures are induced by different TLR ligands (Amit et al., 2009). Therefore, the complexity of innate signatures induced by YFV-17D is likely to be much greater than that induced by poly ICLC. Caskey et al. (2011) appropriately point out that pathways such as IL-1 signaling and protein ubiquitination are stimulated by YFV-17D but not poly ICLC. Future studies addressing these issues in more depth and examining gene signatures in isolated DC subsets will pave the way toward the rational development of improved adjuvants.

Better adjuvants are urgently needed, both for new vaccines against challenging diseases like HIV, malaria, and tuberculosis and to increase the efficacy of existing vaccines in the elderly population and immune-compromised individuals.

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