Chapter 4: Conclusions and Future Directions

CONCLUSIONS

In this thesis I have uncovered that antigenic protein can be delivered by viral particles via pseudotransduction. This mechanism of protein delivery is sufficient to generate antigen-specific immune responses in a mouse model. Further, I have found that human DNA is a component of viral particles and acts as a viral ligand to stimulate the host innate immune STING-dependent pathway. The overall significance, innovation, and future directions of these findings are explored in this chapter.

SIGNIFICANCE

Importance of developing novel vaccine approaches. Over the past two centuries, vaccines have led to some of the most important public health achievements including the worldwide eradication of smallpox and near-elimination of polio (Henderson, 2011). In addition to protecting against pathogens, some of the current vaccines can also prevent against certain types of cancers of the liver and cervix (Ungaro et al., 2013). However, there are many pathogens such as HIV, malaria, and tuberculosis, as well as cancers, for which the development of effective vaccines has been difficult. This difficulty is partly due to the fact that intracellular pathogens and cancers require robust antigen-specific $CD8^+$ T cell for protection (Wong and Pamer, 2003). A protein subunit vaccine, which involves the direct administration of a specific protein from a pathogen, is poor at generating $CD8^+$ T cells (Chackerian, 2007). The viral-like particle (VLP) is a type of subunit vaccine, which is much more effective at eliciting cell-mediated immune responses than other subunit vaccines. They are constructed from few viral proteins and lack a viral genome. Despite their simplified nature, VLPs infect cells in a highly immunostimulatory fashion, thereby generating antigen-specific $CD8^+$ T responses to their viral proteins. In addition, VLPs are a safer alternative than live-attenuated vaccines composed of modified forms of entire microbes with reduced pathogenicity, which can be lethal to immunocompromised patients and potentially revert to virulent strains. As a consequence, there is a significant need for the development of alternative vaccines to target pathogens for which natural protection is inefficient.

Importance of developing future virus-like particle (VLP) vaccines. One important future line of research involves the development of recombinant VLPs which capitalize on the immunogenic nature of VLPs to deliver the antigens of heterologous pathogens. This type of format could greatly simplify future vaccine design and production. In particular, current vaccine manufacturing is complex, involving diverse culture systems ranging from mammalian cell lines to insect cell lines, and sometimes employing yeast, plant cells, or fertilized chicken eggs (Lua et al., 2014). Significant resources are expended in the development, validation, and elucidation of the mechanism for each type of VLP. Thus, it is important to explore and develop strategies in which a single culture system can generate a broad armament of vaccines against different intracellular pathogens and cancers.

Critical barrier to the use of VLPs as vaccines. Understanding the mechanism by which VLPs exert their immunostimulatory effect has been a barrier to their use. These genome-less vectors do not contains nucleic acids, which are generally thought to be important in stimulating innate immune sensors (Akira et al., 2006, Breckpot et al., 2007, Luban, 2012). Nevertheless, genome-less VLPs do trigger innate responses, presumably through interactions of their viral proteins. The identification of the immunostimulatory VLP proteins and their corresponding innate immune sensing pathways might provide important mechanistic insights with the potential to influence future VLP or adjuvant designs.

Potential to understand mechanisms of vaccination and create improved vaccination platforms. If the proposed aims are achieved, we will have created a highly flexible and simplified method of producing recombinant VLPs with enhanced efficacy. Furthermore, we will also have gained an understanding of the mechanisms by which VLPs exert their immunostimulatory effect and quantified host immune responses. Collectively, successful completion of this proposal could be fundamentally important to future vaccine development by expanding our knowledge of viral mechanisms and host responses to infection.

INNOVATION

The current work is innovative in the following ways. First, we have discovered an immune mechanism used by LVs and VLPs which has identified a previously unknown innate immune sensing pathway. We have found that viral particles encapsulate human DNA, which serves to stimulate the host STING pathway. Secondly, this work explores the use of a dendritic cell (DC)-targeting VLPs, which have not been previously described. The DC-targeting Sindbis virus-derived envelope has been used to specifically direct viral vectors to DCs, which are among the most important antigen-presenting cell of the immune system (Yang et al., 2008, Xiao et al., 2012, Dai et al., 2009). In this work we have developed a highly efficient DC-targeting VLP. Thirdly, we have improved upon the production methods for producing recombinant VLPs delivering heterologous antigens from human cell lines. Currently, recombinant VLPs are made by fusing heterologous antigens to the viral proteins of the VLP (Roose et al., 2013, Stoute et al., 1997, Neirynck et al., 1999, Vietheer et al., 2007, Arora et al., 2013, Kaczmarczyk et al., 2011, Gheysen et al., 1989). However, the immunodominant epitope may not be known or more than one may be required for protection. Furthermore, fusion of epitopes to viral proteins may disrupt the ability of VLPs to self-assemble into particles. In this thesis we provide an innovative and simple method for incorporating whole heterologous proteins into VLPs. Lastly, this work has generated easy ways to produce immunostimulatory vectors and investigate new fundamentally important mechanisms of immunity.

FUTURE DIRECTIONS

These interesting findings lead to further questions about pseudotransduction and human DNA-mediated immune stimulation.

1) How does heterologous protein and human DNA get encapsulated into viral particles? Is the mechanism of encapsulation passive or directed?
2) What is the characterization of human nucleic acids in the viral particles? What is the genetic landscape of dsDNA represented in these viral particles? Is the whole human genome represented randomly or non-randomly? Are there ssDNA as well, which may be

important to immune stimulation? Are there human RNA molecules which may also have an immunostimulatory function? Is this process of protein and DNA encapsulation generalizable to other viruses and cell types? Our results suggest this process is not specific to the transfection process or 293T cells. Does the viral envelope of HIV also induce DC activation similarily to the viral envelope from VSV and Sindbis virus?

3) Viral particles are known to represent a heterogeneous population of particles. Do the particles differ by heterologous protein and human DNA composition? Are there methods to separate particles based on these components?
4) What is the innate immune sensor that detects human DNA released via viral fusion into the cytoplasm? Various cytosolic DNA sensors signal through the STING-dependent pathway. It is unclear which of the putative DNA cytosolic sensors are involved in LV detection. In particular, is the dsDNA cytosolic sensor cGAS involved in LV or VLP-mediated DC stimulation?
5) It appears that viral fusion itself also stimulates DCs in a STING-independent method. What is the mechanism of this STING-independent process?
6) What role does cell death play in LV-mediated immune stimulation? Do LV-treated cells die by apoptosis? If so, is autophagy involved?
7) Can DC-targeting VLPs be used as an effective vaccine? Can these VLPs protect against infectious challenges of intracellular pathogens such as Listeria or LCMV, which required potent memory CD8+ T cell responses?

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