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Summary of the workshop "Improving chances for successful clinical outcomes with better preclinical models"

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ABSTRACT

In order to avoid expensive clinical failures, better and more predictive animal models of vaccine efficacy are needed to screen *Shigella* and ETEC vaccine candidates for protective efficacy. The 2016 Vaccines Against *Shigella* and ETEC (VASE) Conference included a workshop focused on the strengths and weaknesses of current models, particularly in terms of the correlation to vaccine efficacy in human clinical trials. Workshop presenters shared information on existing preclinical animal models for assessing the immunogenicity and protective efficacy of *Shigella* and ETEC vaccines. The presentations were followed by a discussion about how to best utilize these models, how the models can be improved, and best practices for *Shigella* and ETEC vaccine developers. The workshop concluded with three major recommendations for the field: (1) develop better and more consistent reagents for animal studies and make them widely available, (2) prioritize harmonization of animal models and immunology assays, and (3) develop preclinical correlates of protection, which will be key in selecting the best vaccine candidates for further clinical development.

1. Introduction

Preclinical models of *Shigella* and Enterotoxigenic *Escherichia coli* (ETEC) infection hold the promise of elucidating immune mechanisms of protection and predicting vaccine efficacy in humans. Although the vaccine field for *Shigella* and ETEC benefits from several animal models, none have shown direct correlation with results from the clinical setting. However, advances in the practical animal methods and immunology assays as well as a deeper understanding of the host immune system may provide additional insight to guide the rational design of vaccines and accurately predict future clinical successes.

This workshop at the 2016 VASE Conference was designed to share the latest developments in preclinical models being employed for vaccine evaluation and to facilitate discussion on the utilization of these models in the development and preclinical evaluation of *Shigella* and ETEC vaccines. The workshop began with presentations by Kristen Clarkson (Walter Reed Army Institute of Research [WRAIR]) on guinea pig models for *Shigella*, Dr. Weiping Zhang (Kansas State University) on porcine and rabbit ETEC models, and Drs. Milton Maciel, Jr. (Naval Medical Research Center) and Mark Smith (WRAIR) on non-human primate models for *Shigella* and ETEC. Following presentations on the methods and recent results, workshop attendees participated in a guided discussion on how these models may be employed and agreed on recommendations for best practices for the field.

Animal models that mimic human disease are highly desirable tools for studying *Shigella* and ETEC pathogenesis and vaccine efficacy. Essential characteristics of pathogenic *Shigella* are the ability to invade the human intestinal mucosal epithelium, replicate intracellularly, and spread intercellularly, all of which ultimately lead to shigellosis. Although there are several animal models that involve some of the pathogen's key virulence traits that have been used to study the pathogenesis and immunogenicity of *Shigella* spp. and *Shigella* vaccines, the models most often used for preclinical

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immunogenicity and efficacy evaluation are the non-human primate, mouse, and guinea pig models. Similarly, in the field of ETEC vaccine development, several models have been developed over the past decade, but the non-human primate and pig models may be the most useful in understanding the efficacy of vaccines. The next three sections highlight key points addressed in the workshop presentations.

2. Guinea pig models for *Shigella* vaccine efficacy studies

The guinea pig keratoconjunctivitis model [12], in which ocularly challenged animals develop an inflammatory disease of the conjunctiva, has been used to evaluate several *Shigella* vaccine candidates ranging from live attenuated to inactivated whole cell and subunit approaches. Infection is initiated by placing approximately 10^8 bacteria onto the eye and scoring daily for conjunctivitis and keratitis and the development of corneal ulcers and purulence [7]. The model has been helpful in down-selecting vaccine formulations and determining the correlation of ocular IgA titers, specific for *Shigella* lipopolysaccharide (LPS), and protection from disease [10]. Studies presented at the workshop demonstrated that previous ocular infection confers resistance to subsequent challenges with the homologous *Shigella* serotype, building on previous observations with a non-human primate model [4]. The LPS-specific ocular IgA titers in the veteran guinea pig group correlated with protection (Spearman $r = -0.9368$; $p < 0.0001$, suggesting that local LPS-specific immunity contributes to local defenses.

An alternative to the guinea pig keratoconjunctivitis model is the intestinal model. This model was previously shown to induce rectocolitis in guinea pigs weighing less than 280 g [5]. However, in most vaccine efficacy studies, animals grow to greater weights (>375 g) due to immunization regimens. Therefore, to be useful in studies with prolonged immunization and challenge timelines, the model was refined to induce rectocolitis in larger, older guinea pigs. New technical aspects included adjusting the bacterial growth conditions, increasing the inoculum volume and use of a longer, rectal catheter to instill the inoculum farther into the large intestine. The intrarectal challenge dose was optimized for *S. flexneri* 2a, *S. flexneri* 3a, and *S. sonnei*. Each serotype was directly compared to a serotype-matched avirulent *Shigella* strain. Varying challenge doses were evaluated in animals weighing ≥ 400 g to achieve an 80% attack rate. A challenge dose of 5×10^{10} cfu was found to be the optimal dose for *S. sonnei* and *S. flexneri* 2a, whereas *S. flexneri* 3a required a half-log fewer bacteria.

During dose-optimization studies, multiple disease parameters (blood, mucous, diarrhea, inflammation, and weight change) were examined to fully characterize the disease. In addition, animals were necropsied to examine multiple sections of the large intestine and rectum to characterize and determine the severity of pathology caused by infection. All animals infected with virulent strains of *Shigella* exhibited some, if not all, outward signs of disease and lost 5–10% of their body weight 24 h post-challenge. Peak disease for all three serotypes was at 48 h post-infection. Gross and histologic evaluation of the large intestine and rectum revealed localized rectocolitis characterized by intracellular bacteria in the colonic epithelium, epithelial loss, an inflammatory infiltrate composed of polymorphonuclear inflammatory cells (heterophils), edema, and hemorrhage. In summary, intrarectal challenge of guinea pigs with virulent *Shigella spp*. is able to induce reproducible rectocolitis that in many aspects mimics human disease. The refined model is relevant for use in *Shigella* vaccine efficacy studies and should be further investigated using other *Shigella* vaccine formulations delivered either parenterally or mucosally.

Both guinea pig models have similarities to the human shigellosis in that the disease is characterized by mucous, neutrophils, tissue integrity loss, ulceration, and inflammation. The guinea pig rectocolitis model targets the same colonic tissue as the human model but the challenge dose ($\sim 10^{10}$) is high as compared to the challenge dose for humans ($\sim 10^3$). Future method refinements and a more comprehensive understanding of the guinea pig immune system and response to infection may provide the necessary insight to predict clinical successes.

3. Pig and rabbit models for ETEC vaccines

One of the key challenges in developing ETEC vaccines is the lack of a suitable animal model to evaluate efficacy of candidates prior to human volunteer studies. An ideal model would use animals that have a normal and patent intestine, while its immune system would be functioning and uncompromised, and would naturally develop diarrhea similar to the human patients in intensity and duration in response to ETEC infection. Mice, which are commonly used in antigen immunogenicity studies [13], are not suitable for ETEC vaccine efficacy studies because mice do not naturally develop diarrhea after ETEC infection. In contrast, rabbits, pigs, and perhaps non-human primates develop diarrhea after infection, thus are potential models for ETEC vaccine development.

Pigs, particularly young pigs, like humans, are naturally susceptible to ETEC infection and develop clinical diarrhea after infection. More importantly, biological relevance between the colonization of ETEC bacteria expressing specific fimbrial adhesins in small intestines of pigs producing receptors specific to porcine ETEC adhesins, and the development of clinical diarrhea in these pigs, has been well established. Thus, natural resistance due to the lack of host receptors can be eliminated, leading to unambiguous evaluation of protective efficacy of ETEC vaccine candidates. Additionally, pigs are more similar to humans in physiology, organ development, and immune systems than other animals commonly used in biomedical research.

Like humans, pigs develop immunity after natural infection or experimental vaccination, and such derived immunity protects against subsequent ETEC infection. Pigs exposed to ETEC bacteria or immunized with ETEC toxin and fimbrial adhesin antigens develop IgG and IgA antibodies specific to ETEC toxins and adhesins, and these pigs are protected when challenged with homologous ETEC strains. That makes pigs a highly relevant animal model to assess ETEC vaccine candidacy.

While pigs and humans express the same receptors for ETEC enterotoxins, heat-labile toxin (LT) and heat-stable toxin (STa), they produce host-specific receptors for ETEC fimbrial adhesins. Pigs may not produce the same receptors for adhesins of human-type ETEC bacteria, and cannot be effectively colonized by human ETEC bacteria. Therefore, while the pig model is ideal to measure protective efficacy of anti-toxin immunity against ETEC diarrhea and to examine adhesin antigens for induction of host immune responses, it becomes less effective to assess efficacy of immunity derived from adhesin antigens against ETEC colonization in small intestines.

Rabbits, a model only briefly discussed in the workshop, can be colonized by human ETEC strains, although they do not react well to STa toxin. That makes rabbits useful for the evaluation of anti-adhesin immunity against ETEC colonization in the gastrointestinal tract, but not for protection from toxin-related diarrhea. By using the pig model to measure efficacy against enterotoxicity and diarrhea and using the rabbit model to assess efficacy against ETEC colonization, we can unambiguously evaluate efficacy of an ETEC vaccine candidate against ETEC diarrhea.

4. *Aotus nancymaae* model for *Shigella* and ETEC vaccines

A. nancymaae, also known as the owl monkey, is a new world primate found in the jungles of Central and South America. They

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