VACCINE ADJUVANTS AND DELIVERY SYSTEMS

Edited by

MANMOHAN SINGH, M. Pharm., Ph.D.
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Emeryville, California

WILEY-INTERSCIENCE

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PREFACE

Prevention of infectious diseases, allergies, malignancies, fertility, and immune disorders using vaccination technologies has been explored extensively in the past decade. Also, the discovery of new antigens through the host genome, which are predominantly recombinant proteins, will require the use of potent immunopotentiators and suitable delivery systems to engender strong responses.

Alum remains the most common adjuvant used in the vaccine market globally. Apart from its safety profile, its use had expanded due to the lack of availability of a suitable alternative. In the last few years, the awareness of how some vaccine adjuvants work has led to a dramatic increase of focus in this area. Whether through activation of innate immune responses or delivery to the targeted site, these novel adjuvant formulations can now be better characterized and optimized for their function. Formulations can now be designed to induce both cellular and humoral responses. Local responses using the nasal and oral routes can now be generated using selective mucosal adjuvants. Evaluation of synergistic effects and repeated use are also being explored. However, these new technologies will have to demonstrate a safety profile that is acceptable for mass immunization and prophylactic use.

This book highlights some of these newly emerging vaccine technologies, some of which will be part of licensed products in the near future. The book evaluates in depth all factors that govern induction of an optimal immune response. Chapters on adjuvant history, antigen presentation, mechanism of action, and the safety profile build a sound base for addressing specific vaccine formulation issues. Detailed descriptions of all leading vaccine formulations and technologies, together with their limitations, should help both researchers and students to enhance their understanding of these technologies. Some of these formulations are purely delivery systems; others comprise immune

potentiators with or without delivery systems. The book also has chapters on clinical and nonclinical safety evaluation of vaccine formulations which should serve as prerequisites in moving vaccine research from preclinical to clinical testing. Overall, the book highlights most recent advances in the field of adjuvant and vaccine research.

MANMOHAN SINGH

1

DEVELOPMENT OF VACCINE ADJUVANTS: A HISTORICAL PERSPECTIVE

GARY OTT AND GARY VAN NEST

1.1 INTRODUCTION

Since the earliest attempts to raise significant immune responses against nonliving agents, investigators have tried to identify useful additives that can be combined with antigens to enhance immune responses. Such immune-enhancing additives are known as *adjuvants*. Virtually all adjuvant systems developed to date have focused on one of two mechanisms: specific immune activation or the delivery–depot effect. Although many adjuvant systems have been developed and tested in preclinical models, few have actually proved useful for human vaccines. The primary limitations for the use of new adjuvant systems with human vaccines revolve around safety issues. Whereas the toxicity of adjuvants has been reduced systematically through research and development efforts over the last 80 years, the safety barriers presented by regulatory and liability issues have continued to increase. Adjuvants to be used with prophylactic vaccines in normal, healthy populations need to have virtually pristine safety profiles. The fact that most vaccines today are given to infants or children heightens the safety concerns of vaccine adjuvants.

In this chapter we review the history of vaccine adjuvant development from the beginning studies of the early twentieth century through to the present day. We recognize four periods of adjuvant development: (1) the initial

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development of adjuvants for toxoid vaccines from the 1920s to the 1940s, (2) the broadened use of oils and aluminum adjuvants from the 1940s to the 1970s, (3) the development of synthetic adjuvants and second-generation delivery–depot systems from the 1970s to the 1990s, and (4) the development of rational receptor-associated adjuvants that active the innate immune system from the 1990s until the present day. We provide perspectives in the areas of work in preclinical systems, clinical evaluation and the use of adjuvants, and the interplay between immunology and adjuvant development in each of these periods.

1.2 INITIAL DEVELOPMENT OF ADJUVANTS FOR TOXOID VACCINES: 1920s–1940s

Some of the earliest studies leading to the development of adjuvants for active vaccines involved live [1] or killed bacterial vaccines in which the antigen and immune-stimulating agents were both provided by the bacteria [2,3]. Protection against diphtheria by passive transfer of horse antidiphtheria antiseria was a Nobel Prize–winning advance by von Behring [4]. The concept of an active subunit vaccine was first demonstrated in 1907 by Smith, who demonstrated that administration of toxin/antitoxin in immunoprecipitating ratios could provide protection, and von Behring used this approach in people with some success in the period 1910–1920 [4]. Addition of oil or lanolin with killed salmonella is the first documented study with a delivery–depot substance used with a killed bacterial vaccine [5]. Adjuvant research began in earnest with the development of diphtheria subunit toxoid [6] vaccines due to the weak immunogenicity observed with these vaccines [7–9]. As noted by Freund: "Interest in promoting antibody formation by addition of unrelated substances to antigens has never been lacking" [10]. Substances such as agar, tapioca, lecithin starch oil, saponin, salts of calcium and magnesium, killed *Salmonella typhi*, and even bread crumbs were tested [6,11,12].

The most significant vaccine adjuvants to be developed are the aluminum salt adjuvants: generically, but not correctly, referred to as *alums*. The first alum-adjuvanted vaccine was formulated by coprecipitation of diphtheria toxoid dissolved in carbonate buffer (pH 8.0) with aluminum (a purification trick), resulting in a coprecipitate of aluminum hydroxide and diphtheria toxoid [13,14]. The alum adjuvant was developed on the basis of faster and higher antitoxoid antibody responses in guinea pigs. The results of human trials with diphtheria toxoid precipitated with alum were published as early as 1934 [15]. Coprecipitated alum–toxoid nearly eradicated diphtheria in Canada in the 1920s and 1930s. Successful trials with tetanus toxoid were completed in the same time frame [16]. However, some early alum formulations showed poor reproducibility, and results of failed clinical trials were also published by Volk [17]. The alternative approach of adsorbing antigen to the surface of "naked" alum particles was demonstrated as early as 1931 [18] and later came

into common use. Only occasional and moderate toxicities were reported with these early alum–toxoid vaccines. The levels of toxicity seen were deemed acceptable given the dramatic decreases in diphtheria and tetanus disease resulting from use of the vaccines.

While the low-toxicity depot approach with alum went forward in clinical applications, efforts were made to generate more potent vaccines using several approaches. One such approach was the use of toxin–antitoxin mixtures [19]. Another approach involved work with tuberculosis (TB) vaccines which demonstrated that the inflammation induced by TB could enhance immune responses to other antigens. As early as 1924, Lewis noted that intraperitoneal injection of live TB a few days before immunization with a variety of antigens dramatically increased antibody responses to those antigens [20]. Presentation of antigen at inflammatory TB foci resulted in elevated antibody titers [21]. These observations pushed forward the immunostimulatory adjuvant approach, which in the 1930s meant the generation of inflammation.

The next advance in adjuvant development involved the combination of killed tubercle with oils. Initial combinations of killed tubercle with paraffin oil produced sensitization to TB but no increased protection from disease [22,23]. Freund demonstrated similar increased antibody responses using live TB with oils. Freund made two jumps in the technology in the 1930s with the substitution of killed TB for live TB and the use of a water-in-oil emulsion [24], inspired by repository formulation techniques being used at the time [10]. The water-in-oil emulsion was formed by the mixture of one volume of 10% Arlacel A (mannide monooleate) and 90% mineral oil with one volume of antigen solution. This system became the standard for adjuvant activity when Freund demonstrated that the emulsion without killed TB was almost as potent as the emulsion with killed TB when used as an adjuvant with diphtheria toxoid and far exceeded the potency of an alum–toxoid formulation [10]. These emulsions went on to become the standard potent adjuvant systems used in preclinical settings and became known as *complete Freund's adjuvant* (CFA, with killed TB) and *incomplete Freund's adjuvant* (IFA, without TB). The emulsion adjuvant was shown to have activity with a variety of antigens, including those from Japanese encephalitis and influenza virus being developed in the same period [25,26]. Water-in-oil emulsion without TB was tested in early human trials with influenza vaccine and demonstrated faster and higher antibody responses than those of vaccine alone [27].

By the mid-1940s, two major adjuvant systems had emerged: the low-reactogenic, modestly effective, and difficult-to-reproduce alum systems, and the new, more potent water-in-oil emulsion systems. It was postulated that alum worked by means of a slow-release depot system [14]. Freund attributed the activity of the water-in-oil emulsion in some part to extended antigen presentation [10]. In this era, adjuvant discovery scientists appeared to be closely involved with immunologists of the day, with adjuvant mechanisms contributing to immunological theory.