Vaccine xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Molecular signatures of vaccine adjuvants

Thorunn Olafsdottir, Madelene Lindqvist, Ali M. Harandi*

Department of Microbiology and Immunology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Medicinaregatan 7A, Gothenburg, Sweden

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Vaccine adjuvants
Systems biology
Omics
Mode of action of adjuvants

ABSTRACT

Mass vaccination has saved millions of human lives and improved the quality of life in both developing and developed countries. The emergence of new pathogens and inadequate protection conferred by some of the existing vaccines such as vaccines for tuberculosis, influenza and pertussis especially in certain age groups have resulted in a move from empirically developed vaccines toward more pathogen tailored and rationally engineered vaccines. A deeper understanding of the interaction of innate and adaptive immunity at molecular level enables the development of vaccines that selectively target certain type of immune responses without excessive reactogenicity. Adjuvants constitute an imperative element of modern vaccines. Although a variety of candidate adjuvants have been evaluated in the past few decades, only a limited number of vaccine adjuvants are currently available for human use. A better understanding of the mode of action of adjuvants is pivotal to harness the potential of existing and new adjuvants in shaping a desired immune response. Recent advancement in systems biology powered by the emerging cutting edge omics technology has led to the identification of molecular signatures rapidly induced after vaccination in the blood that correlate and predict a later protective immune response or vaccine safety. This can pave ways to prospectively determine the potency and safety of vaccines and adjuvants. This review is intended to highlight the importance of big data analysis in advancing our understanding of the mechanisms of actions of adjuvants to inform rational development of future human vaccines.

1. Secrets to be unraveled by omics

Conventional immunological approaches such as serology and cellular immunology have been successfully employed to study immune responses triggered by human vaccines, and as such confer significant insights into the vaccine-induced immune responses. Systems biology is an emerging inter-disciplinary field of studying complex interactions within biological systems in a holistic manner by the means of mathematical and computational modeling. Integration of multiple layers of data, derived from distinct 'omics' such as transcriptomics, proteomics, and metabolomics using various platforms has the potential to provide an in depth understanding of the complex mechanisms underlying immune responses induced by vaccines and adjuvants. Further, such multi-omics approach offers an unprecedented opportunity to identify early signatures/biomarkers predictive of magnitude, quality and/or longevity of the vaccine/adjuvant-induced adaptive immune responses as well as efficacy and safety of vaccines.

Recently, systems biology approach has been employed in vaccine research where data obtained from different omics and conventional immunological read outs were integrated to decipher the mode of actions of human vaccines. The first pioneering study of the vaccine-induced immunity through a systems biology approach was conducted in healthy adults vaccinated with the live attenuated yellow fever vaccine YF-17D, where early predictive signatures of later CD8 T cell- and B cell-responses were identified [1]. The study of yellow fever vaccine has been elegantly followed up by a large-scale study, analyzing blood transcriptome profile of five human vaccines, addressing the key question of whether there are universal predictors of vaccine efficacy. Distinct transcriptional signatures were found to correlate with vaccine-specific antibody responses, suggestive of vaccine-specific responses, rather than a universal signature across vaccine types [2].

The snap shot of migrating cells and molecules observed in the blood can be complemented with the analysis of the immune response in the lymphoid tissues. Animal models can provide the opportunity of profiling biomarkers in the lymphoid and mucosal tissues in addition to the blood and as such can help identifying blood biomarkers that reflect immune response status in the lymphoid tissues and target organs. The importance of evaluating transcriptional changes induced by adjuvants *in vivo* has been

* Corresponding author. Tel.: +46 31 7786229.
E-mail address: ali.harandi@microbio.gu.se (A.M. Harandi).

http://dx.doi.org/10.1016/j.vaccine.2015.04.099

recently shown for the two human adjuvants Alum and MF59. While these two adjuvants did not induce any appreciable transcriptional changes in splenocytes *in vitro*, intramuscular (i.m.) injection triggered significant changes in gene expression at the site of injection [3]. Nevertheless, two recent papers have reported conflicting results on how well genomic responses in mice could mimic that of human. Seok et al. [4] reported a poor correlation between mouse and human transcriptomics profiles in inflammatory diseases. A report from Takao et al. [5], on the other hand, reported that mouse models closely captured the transcriptomics profile of humans when the same datasets were re-analyzed using a different statistical analysis approach. Seok et al. selected genes for correlation analysis based solely on genes that were found differentially expressed in humans whereas Takao et al. based their analysis on genes that were differentially expressed in both mice and humans Further differences in the methodology of the two papers included the choice of correlation analysis methods and different treatments of time-course data, resulting in two contradicting conclusions derived from the same original datasets. This exemplifies how the choice of methodologies used for the analysis of transcriptomics data can result in different conclusions, emphasizing the importance of a careful selection of data analysis approaches.

Systems biology has recently been used to study the mode of actions of vaccine adjuvants. Below we will discuss a handful of publications currently available on the transcriptome profiling following *in vivo* administration of adjuvants in animal models (summarized in Table 1).

2. Mode of actions and signatures of adjuvants used in human vaccines

With some billion doses of alum-adjuvanted vaccines given to humans since its discovery in 1920s, alum is the most widely used adjuvant in human vaccines and as such is often included as a benchmark in vaccine adjuvant research. Nevertheless, its multifaceted mechanisms of action have only recently been studied [6]. These studies indicate that alum adjuvanticity is mediated by an increase in antigen uptake, induction of danger signals, and recruitment of numerous types of immune cells [7]. The lack of uniformity in the type of alum and immunization protocols used across different mode of action studies has however yielded different results such as the role of NALP3 inflammasome in alum mediated adjuvanticity [8,9].

MF59 developed by Novartis (ex-Chiron) represents the first adjuvant used in human vaccines in the post-alum era. MF59 is an oil-in-water emulsion containing the fully metabolizable oil squalene, Tween 80 (a water-soluble surfactant) and Span 85 (an oil-soluble surfactant) that induces a strong antibody response [10]. MF59 has been shown to enhance the diversity and affinity of the antibody response to influenza vaccination in humans [11]. Extensive immunological and transcriptome analyses have been performed on MF59 to evaluate its mode of action, making it one of the best characterized adjuvants so far [3,10,12,13]. The mode of action of MF59 has been compared with that of alum, providing valuable information on common as well as unique features of these two antibody inducing adjuvants [3,13]. Whole transcriptome analysis on mouse quadriceps following i.m. injection of MF59 or alum revealed that MF59 induces transcriptional changes of almost three-times as many genes as alum at the injection site and that the expression of only 34 genes were exclusively changed by alum [13]. Common genes whose expression significantly changed by both of the two adjuvants included cytokines and cytokine receptors genes together with genes involved in antigen processing and presentation. However, MF59 was found to induce a stronger and quicker gene expression compared to alum for the majority of the common up-regulated genes. Among exclusive genes whose expression up-regulated in the muscle fibers by MF59 were early biomarkers such as the transcription factor JunB (involved in regulating gene activities) and pentraxin 3 (involved in inflammatory responses). Similar to MF59 alone, MF59 in combination with the flu antigen was shown to be a strong modulator of transcripts locally in the muscle with no significant changes in the draining lymph nodes [3]. Transcriptional profiles of MF59 indicated that it induced IFN-type I independent adjuvanticity that was confirmed by the finding that *in vivo* administration of IFN- type I receptor blocking antibody did not influence the immune enhancing effect of MF59.

Adjuvant systems 03 and 04 (AS03 and AS04) developed by GSK are included in few licensed human vaccines [14–17]. AS03 is a squalene oil in water emulsion containing α-tocopherol (a form of vitamin E) and polysorbate 80. AS04 consists of alum and the TLR 4 agonist monophosphoryl lipid A. Both adjuvants were reported to activate NF-κB, a master transcription factor of innate immune response, in a transgenic NF-κB luciferase reporter mouse model [18,19]. The NF-κB activation was restricted to the site of injection and the draining lymph nodes and no activation was observed in the remote draining lymph nodes demonstrating a localized response to the adjuvants. Similar to another oil-in-water emulsion based adjuvant MF59, AS03 induced expression of higher number of genes of the immune cell recruiting chemokines and pro-inflammatory cytokines than alum [18].

3. Mode of actions of exploratory adjuvants in or close to clinic

The potential of the TLR ligands as vaccine adjuvants has been extensively explored [20–23]. The TLR9 agonist unmethylated cytidine–phosphate–guanosine (CpG) oligodeoxynucleotides are much studied adjuvant candidates both in preclinical and clinical settings [24]. Microarray analysis of spleen cells following intraperitoneal (i.p.) injection of mice with CpG identified several major inducers of gene network regulation involved in inflammatory responses, including TNFa, IL1a, IL1b and IFNg at 3 h [25]. This was followed by up-regulation of genes for suppressor of cytokine signaling 1 (SOC1) and SOC3 molecules along with IL-10 at 24 h with a possible role in controlling the inflammatory response. In a comparative mode of action study of CpG, MF59 and alum, a set of "adjuvant core responsive genes" was identified at the site of injection following i.m. immunization [13]. Functional analysis on these common genes revealed enrichment of cytokine-cytokine receptor interactions, host-pathogen interaction and defense immunity protein activity. The TLR 7/8 ligand, Resiquimod R848 and the TLR1/2 ligand Pam3CSK4 were also evaluated following i.m. administration in mice [3]. R848 was shown to strongly modulate interferon-related genes as well as a broad activation in the draining lymph nodes observed in cytokine and interferon-related genes. However, much fewer interferon related genes were induced by Pam3CSK4 and the effect on the draining lymph nodes was only modest compared to that of R848 [3]. It is however noteworthy that dendritic cells in mice and human possess distinct patterns of TLR expression that can limit the translation of data from mice to humans at least for TLR-based adjuvants [26]. Recently, transcriptome profiles of the TLR ligands (TLR-L) MPL (TLR4-L), Resiquimod R848 (TLR7/8-L) and CpG (TLR9-L) were compared in rhesus macaques [27]. All TLR-Ls induced rapid and robust expansion of neutrophils in the blood as well as expansion of CD14+ monocytes. However, the different TLR adjuvants also showed distinct signatures of early innate responses in blood and the draining lymph nodes with the main differences observed in their impact

Please cite this article in press as: Olafsdottir T, et al. Molecular signatures of vaccine adjuvants. Vaccine (2015), http://dx.doi.org/10.1016/j.vaccine.2015.04.099