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Review

Applications of chemokines as adjuvants for vaccine immunotherapy

Teena Mohan, Wandi Zhu, Ye Wang, Bao-Zhong Wang*

Center for Inflammation, Immunity and Infection, Institute for Biomedical Sciences, Georgia State University, 100 Piedmont Ave SE, Atlanta, GA 30303, USA

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ABSTRACT

Vaccinations are expected to aid in building immunity against pathogens. This objective often requires the addition of an adjuvant with certain vaccine formulations containing weakly immunogenic antigens. Adjuvants can improve antigen processing, presentation, and recognition, thereby improving the immunogenicity of a vaccine by simulating and eliciting an immune response. Chemokines are a group of small chemoattractant proteins that are essential regulators of the immune system. They are involved in almost every aspect of tumorigenesis, antitumor immunity, and antimicrobial activity and also play a critical role in regulating innate and adaptive immune responses. More recently, chemokines have been used as vaccine adjuvants due to their ability to modulate lymphocyte development, priming and effector functions, and enhance protective immunity. Chemokines that are produced naturally by the body's own immune system could serve as potentially safer and more reliable adjuvant options versus synthetic adjuvants. This review will primarily focus on chemokines and their immunomodulatory activities against various infectious diseases and cancers.

1. Introduction

The goal of vaccination is the generation of strong and long-term protection to a specific pathogen. This is accomplished by eliciting robust immune responses to a targeted antigen which can induce long-term protection against infection. This target antigen could be a laboratory generated or altered pathogen, virus, bacteria, protein, or other foreign substance. These antigens can trigger responses from the host immune system that result in long-term immunity in the process. Certain vaccines, often containing live-attenuated antigens, are effective because they can stimulate the innate immunity to cascade into subsequent adaptive immune responses capable of clearing the targeted pathogens (Hoebe et al., 2004). New developments in the field of vaccinology include DNA vaccines, novel peptides, recombinant viruses and proteins, and conjugates, all of which have been introduced commercially. This new generation of vaccines is safer and has fewer negative reactions or side effects than previous generations of vaccines made from live or whole-inactivated organisms. Conversely, these new advancements are sometimes not effective when a pathogen's natural infection pathway does not convey long-lasting immunity (Hilleman, 2000). To achieve the desired goal of protective immunity in these cases, the addition of a unique adjuvant to the vaccine is needed.

2. Adjuvants

Adjuvants are molecules or compounds that can enhance immune responses against co-administered antigens. Adjuvants increase immune responses to vaccines, enhance protection against pathogens, increase the speed of primary immune responses, activate the appropriate type of immunity to a given threat, increase the generation of memory responses, and can alter the specificity and breadth of the generated immune responses (Vogel, 1998). Adjuvants can also improve immune responses in populations where responses to vaccines are typically reduced, such as infants, the elderly, and immunocompromised patients. The functional outcomes of enhancing immune responses are manifold, including the allowance for reductions in the quantity of antigen contained in individual vaccine dose. This combination of less antigen and fewer doses can improve the vaccine supply worldwide and reduce the total cost of vaccine production.

There are several adjuvants that are currently in the market or in development but aluminum based adjuvants including aluminum hydroxide, aluminum phosphate, and alum still lead the way. Though alum has been known to cause type-1 hypersensitivity reactions post-administration in a small percentage of patients, it is among the few adjuvants that have been approved by the Food and Drug Administration (FDA) for human use (Gupta et al., 1995). Aluminum salts that have met FDA approval are aluminum hydroxide, aluminum phosphate, potassium aluminate sulfate, and mixed aluminum compounds. A significant number of other identified adjuvants are clearly more potent than alum but toxicity is the single most important impediment in introducing most such adjuvants to human use (Edelman, 1980). Some licensed vaccines containing alum or other adjuvants

* Corresponding author.
E-mail address: bwang23@gsu.edu (B.-Z. Wang).

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include: ASO4, comprised of aluminum hydroxide and monophosphoryl lipid A (MPL), is used to treat cervical cancer caused by human papillomavirus (HPV) (Tagliabue and Rappuoli, 2008); AS03, comprised of oil compounds, vitamin E and squalene, is used in an influenza vaccine against H5N1 (Leroux-Roels, 2009). In Europe, MF59 is an adjuvant component of influenza vaccine for elderly patients (Fluad, Novartis Vaccines) (Mulligan et al., 2014) and AS04 (combination of alum and MPL, GlaxoSmithKline) is the adjuvant for some viral vaccines, *e.g.* Hepatitis B virus (HBV) and HPV (Monie et al., 2008).

3. Downsides of synthetic adjuvants

Aluminum based synthetic adjuvants continue to be the dominant adjuvants used. However, these synthetic adjuvants can sometimes trigger unwanted “hyperactive” immune responses, causing the immune system to over-react to antigens found in the vaccine formulations. This increases the probability of disrupting the host’s self-tolerance, leading the immune system to attack its own cells and tissues. It has been established that vaccine induced inflammatory responses can lead to permanent alterations in immune functionality and increase the risk of developing immunological disorders, including autoimmunity and long-term inflammation. Additionally, despite almost 80 years of widespread use, our knowledge behind the mechanisms of aluminum adjuvants is still remarkably limited. An equally concerning lack of data on the pharmacokinetics and toxicology of these compounds also remains problematic. To date, certain new and innovative adjuvants have shown acceptable safety profiles in clinical trials across a variety of applications and in post-licensure experiences, however many of these adjuvants still possess disadvantages such as high-costs, lack of stability and reliability, and most importantly high-toxicity (Tomljenovic and Shaw, 2011; Aomljenovic, 2011).

Chemokines/cytokines, which are produced naturally by the body’s own immune system, could serve as potentially safer and more reliable adjuvant options versus synthetic adjuvants. Vaccine safety, effectiveness, and toxicity could all be improved by using immunostimulators that are naturally non-toxic, have well understood mechanisms, and are adaptable to many types and modes of vaccines. The current review is concerned with the importance of natural and non-toxic adjuvants and their widespread use in the vaccine industry. Published studies on the relevant topics were analyzed and compared to create a composite view of the topic. In this review, we have emphasized the role of various chemokines as adjuvants in the treatment of many diseases and cancers.

4. Cytokines and chemokines

Cytokines are a broad category of small proteins (~5–20 kDa) that are important in cell signaling (Boyaka and McGhee, 2001). Cytokines include chemokines, interferons, interleukins, tumor necrosis factor, and lymphokines. Traditional adjuvants’ mode of action is to stimulate the creation of an environment that promotes immunity. For example, aluminum salts and Freund’s adjuvants have been linked to IFN-γ, IL-2, or IL-12 for establishing both innate and adaptive immunity. Instead of using an adjuvant to induce the production of specific cytokines, these cytokines can now be used directly as adjuvants either alone or in combination with other adjuvants (Grob et al., 1984; Kim et al., 2001).

In one of our recent findings, we also studied a novel cytokine, GIFT4, engineered by fusing GM-CSF and IL-4. We observed that GPI-anchored GIFT4 containing virus-like particles (VLPs) induced higher levels of systemic antibody responses with significantly increased binding avidity and improved neutralizing breadth and potency to a panel of selected strains, as well as higher levels of IgG and IgA at several mucosal sites (Feng et al., 2015).

Chemokines are a family of chemoattractant cytokines which play a vital role in cell migration and in induction of cell movement in response to a chemical gradient by a process known as chemotaxis. Chemokines are small proteins (8–10 kDa) and are present in all vertebrate animals as well as in some microbes including bacteria and viruses, but none have yet been identified in other non-vertebrates (Wiedle et al., 2001). Historically, chemokines have been known by various

names including the SIS family, SIG family, SCY family, the intercrines, and the PF4 superfamily. The action of a chemokine is mediated through chemokine receptors, members of the G protein-coupled receptor (GPCR) family, which can stimulate signal transduction pathways inside the cell when they are activated (Zhang and LiWang, 2014). To date, there are more than 50 chemokines and 18 chemokine receptors identified. These molecules are classified into four families (CC, CXC, C, and CX3C) based on the way the first two conserved cysteine residues are arranged, creating a structural motif (Bachelerie et al., 2014a; Bachelerie et al., 2014b). Chemokines have evolved to become an integral part in the many critical roles involved in regulating innate and adaptive immune responses (Mariani and Panina-Bordignon, 2003). Chemokines modulate lymphocyte development, priming and effector functions, and play a vital role in immune surveillance. Many chemokines have been shown to be effective immunological adjuvants in a variety of model systems, enhancing protection induced by viral, bacterial, and parasitic vaccines (Smit and Lukacs, 2006). These chemokines have also increased various immunological parameters in tumor immunization models and clinical trials (Esche et al., 2005). Chemokines types, binding to their respective receptors and their generalized functions have been demonstrated in Fig. 1.

5. Immunomodulatory studies of chemokines

More recently, chemokines have found a use as vaccine adjuvants, due to their ability to regulate immune responses and enhance the protective immunity (Eo et al., 2001; Lu et al., 1999; Sin et al., 2000). Chemokine adjuvants can modulate the direction and magnitude of induced immune responses generated by DNA, protein, subunit or peptide vaccines. However, most of the chemokine adjuvants are currently struggling with dose-related toxicity issues. Additionally, recombinant chemokines have displayed stability problems, giving them short half-lives and limiting their usefulness as vaccine adjuvants. Some of these limitations can be overcome by utilizing liposome or micro/nanoparticle encapsulation and co-administering chemokine expression vectors with DNA or protein-based vaccines, respectively.

With an array of chemokines and ligands to select from to target on immune cells, it becomes possible to tailor a vaccine’s effects by chemoattracting specific cell types. For example, antigen presenting cell (APCs) targeting approaches have been evaluated for influenza and HIV antigens. In these studies, antigenic delivery with chemokines such as CCL3 have resulted in enhanced immunogenicity and protection against these pathogens. The chemokine CCL3 has demonstrated the ability to attract natural killer (NK) and CD8+ T cells, and has been investigated as an adjuvant for effective vaccines (Allen et al., 2017).

In the CCL3 study, researchers expressed CCL3 together with a truncated HIV-1 Gag antigen in L. plantarum and observed a significant increased recruitment of T cells. Another finding strongly supports that the co-delivery of CCL3 by an adenovirus-based vaccine improves protection from retrovirus infection (Kuczkowska et al., 2015). In the Friend retrovirus (FV) mouse model, CCL3 was co-expressed from adenoviral vectors, together with FV Gag and Env antigens. Env and Gag-Pol antigens coadministered with a vector encoding CCL3 induced higher virus-specific antibody titers and CD4+ T cell responses (Kuczkowska et al., 2015).

In addition, vaccine formulations targeting XCR1 on cross-presenting dendritic cells (DCs) using antigen fused to XCL1, the only known ligand for XCR1, induce protective CD8+ T cell responses against influenza virus and strengthen the role of chemokines in modulating host immunity (Fossum et al., 2015).

Recently, we investigated the effects of GPI-anchored CCL28, a chemokine associated with mucosal surfaces, as an adjuvant with