Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2012, Article ID 831486, 13 pages
doi:10.1155/2012/831486

Research Article

Vaxjo: A Web-Based Vaccine Adjuvant Database and Its Application for Analysis of Vaccine Adjuvants and Their Uses in Vaccine Development

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Received 2 August 2011; Revised 9 December 2011; Accepted 12 December 2011

Academic Editor: Andre Van Wijnen

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Vaccine adjuvants are compounds that enhance host immune responses to co-administered antigens in vaccines. Vaxjo is a web-based central database and analysis system that curates, stores, and analyzes vaccine adjuvants and their usages in vaccine development. Basic information of a vaccine adjuvant stored in Vaxjo includes adjuvant name, components, structure, appearance, storage, preparation, function, safety, and vaccines that use this adjuvant. Reliable references are curated and cited. Bioinformatics scripts are developed and used to link vaccine adjuvants to different adjuvanted vaccines stored in the general VIOLIN vaccine database. Presently, 103 vaccine adjuvants have been curated in Vaxjo. Among these adjuvants, 98 have been used in 384 vaccines stored in VIOLIN against over 81 pathogens, cancers, or allergies. All these vaccine adjuvants are categorized and analyzed based on adjuvant types, pathogens used, and vaccine types. As a use case study of vaccine adjuvants in infectious disease vaccines, the adjuvants used in *Brucella* vaccines are specifically analyzed. A user-friendly web query and visualization interface is developed for interactive vaccine adjuvant search. To support data exchange, the information of vaccine adjuvants is stored in the Vaccine Ontology (VO) in the Web Ontology Language (OWL) format.

1. Introduction

Vaccines are one of the most effective preventative health tools available against infectious diseases, cancer, allergy, and autoimmune diseases. Vaccination aims to generate a strong immune response to the administrated antigen and provide long-term protection against a disease. Frequently, using only the antigen is not enough to stimulate that protective immunity. Vaccine adjuvants are compounds that enhance the specific immune responses against coadministered antigens in vaccines [1, 2]. The word adjuvant comes from the Latin word *adjuvare*, meaning to help or to enhance. The term "adjuvant" was coined by Ramon in the 1920s when they observed that the injection of the diphtheria toxoid along with unrelated substances in horses developed an abscess at the injection site and generated higher specific antibody titers [3, 4]. In 1926, Glenny et al. demonstrated the adjuvant activity of aluminum compounds with diphtheria toxoid absorbed to alum [5]. In 1936, Freund's complete adjuvant (FCA), an emulsion of water and mineral oil containing killed mycobacteria, was developed [6]. Freund's incomplete adjuvant (FIA) that does not have the mycobacteria is less toxic and has been used in many human vaccine formulations [7]. In the 1950s, lipopolysaccharides (LPSs) from Gram-negative bacteria were found to exhibit adjuvant activity [8]. In 1974, muramyldipeptide (MDP) was found to be a mycobacterial component with adjuvant activity in FCA [9]. There is a wide array of adjuvants that are being developed and used, from a variety of sources. Currently, several hundred natural and synthetic compounds are known to have adjuvant activity

[1, 10]. However, the adjuvants that are permitted in licensed vaccines are limited. In the US, alum salts and AS04 (which is composed of aluminum salt and MPL) are licensed for use in humans [11].

Adjuvants can function as immunostimulants, vehicles, and carriers. Cox and Coulter summarize five modes of action of vaccine adjuvants: (1) immunomodulation: the ability of many adjuvants to modify the cytokine network. (2) Presentation: the ability of an adjuvant to preserve the conformational integrity of an antigen and to present the antigen to appropriate immune effector cells. (3) CTL induction: induction of CD8+ cytotoxic T-lymphocyte (CTL) responses. (4) Targeting: the ability of an adjuvant to deliver an immunogen to immune effector cells, generally via antigen presentation cells (APCs). (5) Depot generation: generation of a short-term or long-term depot to give a continuous or pulsed release [12]. The use of vaccine adjuvants allows for a reduction in the number of immunizations or the amount of antigen needed for immunization [13]. Vaccine adjuvants can be administered either parenterally or mucosally.

VIOLIN (http://www.violinet.org/), primarily developed by our group, is the first web-based comprehensive vaccine database and analysis system that targets for vaccine research [14]. Currently, VIOLIN has included more than 2,800 vaccines for over 170 infectious diseases and many noninfectious diseases (e.g., cancers and allergies). Here, a vaccine includes a licensed vaccine or a vaccine construct that is not licensed but has been shown to induce significant protection against a disease in at least one laboratory animal model. Many of these vaccines use vaccine adjuvants. Therefore, it is important to classify them and analyze their differential applications in vaccine development. However, the original VIOLIN database did not support classifications of various vaccine adjuvants or provide detailed vaccine adjuvant information.

While intensive research has been conducted and resulted in identification of many vaccine adjuvants, there is no web-based central resource that allows storage, annotation, comparison, and analysis of vaccine adjuvants and their usages in vaccine development. To address this challenge, we have developed Vaxjo (http://www.violinet.org/vaxjo/), a web-based vaccine adjuvant database and analysis system. Vaxjo stores the information of manually curated vaccine adjuvants and their applications in vaccine development. It also allows bioinformatics analysis and comparison of various vaccine adjuvants. Vaxjo is publically available at http://www.violinet.org/vaxjo/.

2. Methods

2.1. Vaxjo System and Database Design. Vaxjo is implemented using a three-tier architecture built on two HP ProLiant DL380 G6 servers, which run the Redhat Linux operating system (Redhat Enterprise Linux ES 4). Users can submit database or analysis queries through the web. These queries are then processed using PHP/SQL (middle-tier, application server based on Apache) against a MySQL (version 5.0) relational database (back-end, database server). The result of each query is then presented to the user in the web browser. In addition, Vaxjo data curators and reviewers can also submit and review curated vaccine adjuvant data through a web-based Vaxjo data curation system. Two servers are scheduled to regularly backup each other's data. Vaxjo is an integrated program of the VIOLIN vaccine database and analysis [14].

Figure 1 demonstrates the Vaxjo workflow and system design. For each specific vaccine adjuvant, the Vaxjo database contains the following information: (1) detailed reference citation information, for example, authors, journal, title, year, volume, issue, and pages. These references are typically obtained from PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and used to obtain reliable information for vaccine adjuvants. (2) General information of vaccine adjuvants, for example, alternate names, description, stage of vaccine adjuvant development (e.g., preclinical, clinical stage, or licensed), components, preparation, safety, and so forth. This information is manually curated and not available in the general VIOLIN vaccine database originally developed [14]. The manual curation of peer-reviewed journal articles emphasizes the retrieval of vaccine adjuvant information and experimental data associated with vaccine adjuvants. Using an internal script, all the vaccines that are stored in VIOLIN and use a specific vaccine adjuvant can be retrieved and displayed in Vaxjo searching page. A unique Vaccine Ontology (VO) identifier has been assigned to each vaccine adjuvant. The VO IDs used in both the Vaxjo database and the general VIOLIN vaccine database are used as primary keys for linking these two databases. This program allows advanced query and comparison between different vaccine adjuvants and vaccines that use these adjuvants.

2.2. Semiautomatic Annotation of Vaccine Adjuvant Information. A semiautomatic Vaxjo annotation system is developed for vaccine adjuvant curation and analysis by modifying an in-house web-based literature mining and curation system called Limix [14–16] (Figure 2). The interactive Limix data submission and review system (a) allows a data curator to search literature, copy and edit text, and submit data to database and (b) provides user-friendly tools for a data reviewer to review, edit, and approve the curated data on a comprehensive web interface. Upon approval after the critical review by a reviewer, the curated data submitted by a curator will be posted publicly. Limix also features automated reference tracking and management using a version control mechanism similar to that used in Wikipedia (http://www.wikipedia.org/). The system stores all the history changes made to every vaccine adjuvant. A reviewer can compare any two of the history versions to trace the detailed changes made to a vaccine adjuvant. Figure 2 provides a more detailed view of the Limix curation process and reviewing features.

Vaxjo is updated automatically when additional vaccine adjuvants are added. All vaccine adjuvant updates can be found and queried in the Vaxjo website.

2.3. Vaxjo Data Exchange, Transfer, and Download. To facilitate data exchange and transfer, the information of vaccine adjuvants in Vaxjo is now stored in the Vaccine Ontology (VO; http://www.violinet.org/vaccineontology/) [17].

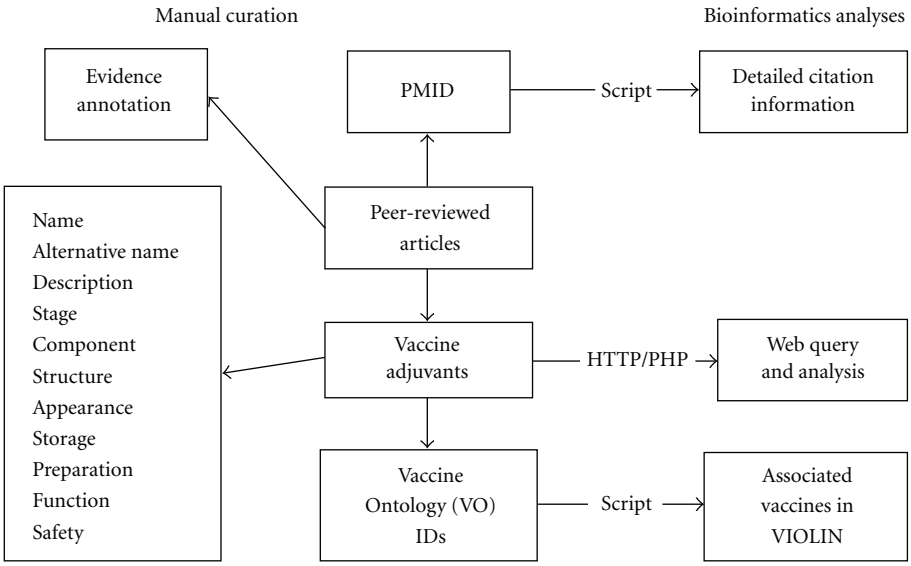

Figure 1: Vaxjo workflow and system design. Manual curation includes peer-reviewed publications from PubMed. A PubMed ID (PMID) is extracted and used by an internal script to retrieve detailed citation information (e.g., authors, journal, and date). The detailed information of a vaccine adjuvant is curated and cited from published studies. Each vaccine adjuvant is assigned with a Vaccine Ontology (VO) ID, which is further used in an internal computational script to obtain the information of relevant adjuvanted vaccines from the general VIOLIN relational database.

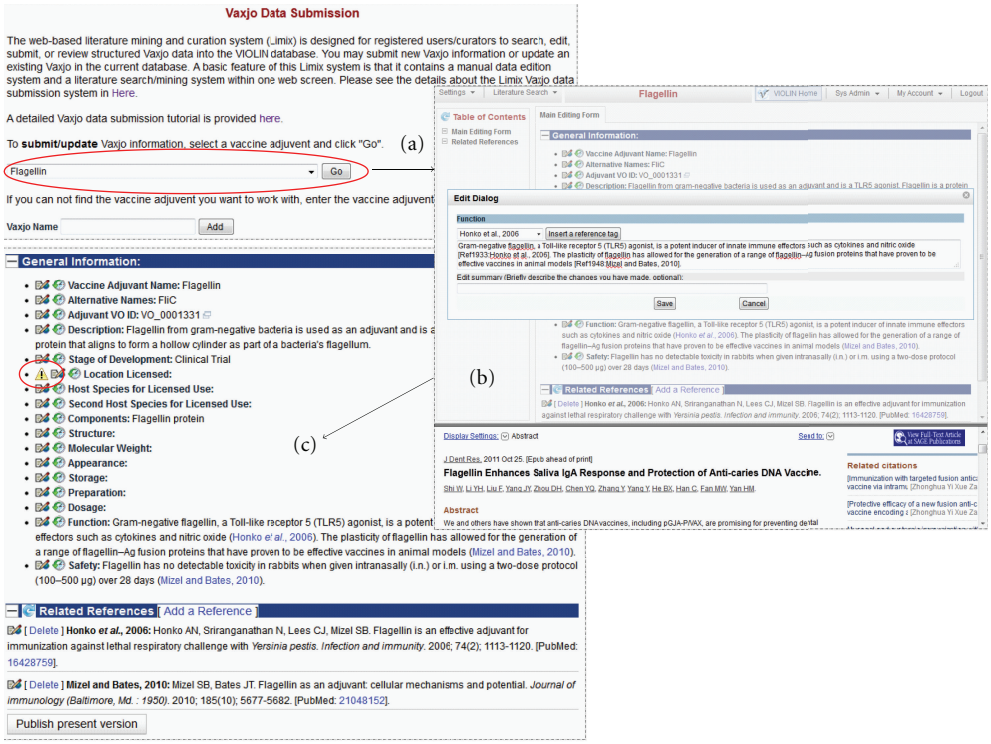

Figure 2: Submission and review processes of vaccine adjuvants into Vaxjo. (a) Selecting an adjuvant from a dropdown list or adding a new adjuvant by entering the name in the field below the dropdown list. (b) Vaxjo submission page, where each section is edited via a popup dialog box that also provides options to insert references when needed. The bottom section of the Vaxjo curation web page includes detailed reference information for a curator to review. (c) Review page. Vaxjo curators review information curated into Vaxjo and, after review, submit the page for publication in Vaxjo.

Table 1: Curated vaccine adjuvants used in at least five vaccines curated in VIOLIN.

Adjuvant type (number of adjuvants)	Adjuvants with >5 vaccines in VIOLIN	Number of vaccines
Mineral salts (13)		154
	Alhydrogel	13
	Aluminum hydroxide vaccine adjuvant	62
	Aluminum phosphate vaccine adjuvant	50
	Aluminum potassium sulfate adjuvant	6
	Aluminum vaccine adjuvant*	14
Emulsions (15)		118
	Freund's complete adjuvant	42
	Freund's incomplete adjuvant	42
	Montanide ISA 720 adjuvant	6
	Ribi vaccine adjuvant	11
Microorganism-derived (18)		73
	Cholera toxin	17
	Cholera toxin B subunit	6
	CpG DNA vaccine adjuvant	12
	LTR192G vaccine adjuvant	6
	MPL adjuvant	10
Synthetic adjuvant (28)		28
Cytokines as adjuvants (9)		20
	IL-12 vaccine adjuvant	9
Tensoactive compounds (5)		12
	Quil-A vaccine adjuvant	5
Combination vaccine adjuvants (6)		9
Particulate antigen delivery systems (9)		11
Carbohydrates (2)		1

Note: *shows those aluminum vaccine adjuvants that were used but without more specific details (e.g., using aluminum hydroxide).

The vaccine adjuvant data stored in VO is machine-readable and can be used for various software programs for data exchange and transfer.

2.4. Vaxjo Data Query and Display. The programming languages PHP and HTML were primarily used to interact with the backend Vaxjo relational database to obtain vaccine adjuvant information. Since Vaxjo has been integrated with the general VIOLIN database system, the Vaxjo data query tool is also able to query the general VIOLIN vaccine database to obtain the information of vaccines that use specific vaccine adjuvants. Meanwhile, the vaccine adjuvant information in the Vaccine Ontology (VO) is queried using internal SPARQL scripts. The VO IDs of different vaccine adjuvants are used as the glue that links the vaccine adjuvants in the Vaxjo database and the general VIOLIN vaccine database.

2.5. Bioinformatics Analysis of the Vaxjo Vaccine Adjuvant Data. The vaccine adjuvants stored in Vaxjo and their applications in development of vaccines stored in the general VIOLIN database were obtained using the web-based Vaxjo query or through internal scripts. A systematic analysis was conducted to classify and compare different characteristics of vaccine adjuvants.

3. Results

3.1. Vaxjo Statistics. Vaxjo is a web-based relational database and analysis system based on a classical three-tier architecture design [15]. Currently, Vaxjo has included 103 vaccine adjuvants. In total, 384 vaccines have been detected to use these vaccine adjuvants. Occasionally, more than one vaccine adjuvant is used in a single vaccine. These 384 vaccines are developed against infections of 76 pathogens, such as *Bacillus anthracis* (anthrax), *Brucella* spp. (brucellosis), influenza virus, and *Plasmodium* spp. (malaria). Vaxjo also stores 15 vaccine adjuvants that have been used for vaccine development against many cancers and allergies.

3.2. Analyses of Vaxjo Vaccine Adjuvants Based on Vaccine Adjuvant Types. With the establishment of the Vaxjo vaccine adjuvant database, we were able to systematically analyze the vaccine adjuvants and their usages in vaccine development. Table 1 shows the types of vaccine adjuvants and lists those

Table 2: Mineral salt adjuvants.

Adjuvant name	Number of Vaccines	Mechanism	Route	Licensed
Adjumer	1	Immunostimulant	Either*	No
Aluminum hydroxide vaccine adjuvant	62	Vehicle/immunostimulant	Parenteral	Yes**
Aluminum phosphate vaccine adjuvant	50	Vehicle/immunostimulant	Parenteral	Yes
Alhydrogel	13	Vehicle/immunostimulant	Either	Yes
Aluminum vaccine adjuvant***	14	Vehicle/immunostimulant	Parenteral	Yes
Aluminum potassium sulfate adjuvant	6	Vehicle/immunostimulant	Parenteral	Yes
Amorphous aluminum hydroxyphosphate sulfate adjuvant (AAHSA)	2	Vehicle/immunostimulant	Parenteral	Yes
Adjumer	1	Immunostimulant	Either	No
Calcium phosphate gel	1	Vehicle	Parenteral	In Europe
Calcium phosphate vaccine adjuvant	1	Vehicle	Parenteral	In Europe
DOC/Alum complex	1	Vehicle/immunostimulant	Parenteral	No
Rehydragel HPA	1	Vehicle	Parenteral	Yes
Rehydragel LV	1	Vehicle	Parenteral	Yes

Notes: *the term "either" means either mucosal or parenteral route. **the term "yes" means that this adjuvant is licensed in the USA. ***shows those aluminum vaccine adjuvants that were used but without more specific details (e.g., using aluminum hydroxide).

adjuvants which have five or more associated vaccines. The adjuvant types are based on the derivation of the adjuvants or the composition of the adjuvants. The largest numbers of vaccine adjuvants are found in microorganism-derived adjuvants (18), synthetic adjuvants (28), and mineral salt adjuvants (13) (Table 1). If the numbers of vaccines using different adjuvants are considered, vaccine adjuvants based on mineral salts, emulsions, and microorganism derivatives have been used in 151, 115, and 73 vaccines, respectively (Table 1). Aluminum hydroxide is the most common adjuvant found, with 62 associated vaccines collected in VIOLIN. Freund's complete and incomplete adjuvants are also commonly used with each being associated with 42 vaccines. Although 28 synthetic adjuvants are curated in Vaxjo, only 28 vaccines listed in VIOLIN use these synthetic adjuvants. Only two of these synthetic vaccine adjuvants collected in Vaxjo are not associated with any vaccines in VIOLIN.

Those commonly used vaccine adjuvants and their associated vaccines are introduced below.

3.2.1. Salt-Based Vaccine Adjuvants. Mineral salt adjuvants are commonly used as aluminum adjuvants that are approved for use in licensed vaccines. Table 2 lists the 13 mineral salt adjuvants in Vaxjo and displays their properties, including the number of associated vaccines to each adjuvant, mechanism of action, route of administration, and whether or not the adjuvant is used in licensed vaccines. Of the mineral salt adjuvants in Vaxjo, 10 are licensed for use in vaccines either in Europe or the United States. Most mineral salt adjuvants act as vehicles for antigens rather than as immunostimulants (Table 2).

Mineral salts, specifically aluminum salts, have been used as adjuvants for decades and are therefore common in vaccine formulations [3, 13]. Alum salts have a depot effect, allowing the antigen to persist in the body so the immune system can react to the antigen and facilitate uptake into antigen-presenting cells (APCs). Aluminum also enhances the antigenicity of some vaccines such as diphtheria and tetanus toxoids [18]. Aluminum-adsorbed diphtheria and tetanus toxoids are more effective than plain fluid toxoids in primary immunization of children. However, there is little difference between plain and adsorbed toxoids for booster immunization [19]. Immune responses to proteins adjuvanted with alum tend to be a T helper cell type 2 (Th2) response [20]. The Chapter 21 of the US Code of Federal Regulations (610.15(a)) limits the amount of aluminum in human vaccines to 0.85 mg/dose. The majority are parenterally used, rather than mucosally. The amount of an aluminum adjuvant in vaccines currently licensed in the US ranges from 0.125 to 0.85 mg/dose [19]. In more than six decades, aluminum vaccine adjuvants usually do not induce serious adverse effects. However, they often induce local reactions such as redness, swelling, and tenderness at the injection site. Occasionally, aluminum adjuvants were found to be associated with severe local reactions such as erythema, subcutaneous nodules, and contact hypersensitivity [19].

3.2.2. Emulsion-Based Vaccine Adjuvants. Emulsion based adjuvants are oil-in-water or water-in-oil emulsions, which work to enable slow release of an antigen at the injection site. Fourteen emulsion-based vaccine adjuvants have been curated and stored in Vaxjo (Table 3). With the exception of the nanoemulsion vaccine adjuvant [21], all the emulsion adjuvants in Vaxjo are delivered via the parenteral route. Nanoemulsion vaccine adjuvant is delivered mucosally [21]. Water-in-oil emulsion adjuvants turn to cause high levels of reactogenicity. Oil-in-water emulsion adjuvants turn to have a low reactogenicity profile. MF59 and AS03 are two licensed

Table 3: Emulsion adjuvants.

Adjuvant name	Number of vaccines	Mechanism	Route	Licensed
Freund's complete adjuvant	42	Immunostimulant	Parenteral	No
Freund's incomplete adjuvant	42	Vehicle	Parenteral	No
Ribi vaccine adjuvant	11	Immunostimulant	Parenteral	No
Montanide ISA 720 adjuvant	6	Vehicle	Parenteral	No
Nanoemulsion vaccine adjuvant	3	Immunostimulant	Mucosal	No
TiterMax gold adjuvant	3	Immunostimulant	Parenteral	No
AF03	1	Immunostimulant	Parenteral	No
AS03	1	Immunostimulant	Parenteral	Licensed
MF59	1	Vehicle	Parenteral	In Europe
Montanide incomplete seppic adjuvant	1	Vehicle	Parenteral	No
Montanide ISA 51	1	Vehicle	Parenteral	No
Specol	1	Immunostimulant	Parenteral	No
SPT (antigen formulation)	1	Vehicle	Parenteral	No
Squalene-based adjuvants	1	Vehicle	Parenteral	No
TiterMax gold adjuvant	3	Immunostimulant	Parenteral	No

oil-in-water emulsion adjuvant used in humans. MF59 is not toxic and prepared with a low content of squalene (4.3% w/w), a biodegradable oil naturally found in plants and animals including humans. In humans, squalene is an intermediate organic compound in the steroid hormone biosynthetic pathway and is a direct synthetic precursor to cholesterol. MF59 induces low reactogenicity at the site of injection. MF59 is able to induce fast priming of antigen-specific CD4+ T-cell responses and to induce strong and long-lasting memory T- and B-cell responses [22]. MF59 has been licensed in more than 20 countries for more than 14 years. It has been used in many licensed vaccines such as Fluad, Focetria, and Aflunov. AS03 (for "Adjuvant System 03") is another squalene-based immunologic adjuvant used in various vaccine products by GlaxoSmithKline (GSK). AS03 is used, for example, in GSK's A/H1N1 pandemic flu vaccine Arepanrix H1N1.

3.2.3. Microorganism-Based Vaccine Adjuvants. Microorganism-based vaccine adjuvants are adjuvants that are derived from microorganisms. Bacteria have an abundance of capacity for stimulating the immune system, and the adjuvants derived from bacteria are abundant. As whole bacteria are generally too toxic to use as an adjuvant, many bacterium-derived vaccine adjuvants are derived from parts of bacteria [1]. Table 4 displays the properties of the 18 microorganism-based adjuvants in Vaxjo. Only two of the microorganism-based adjuvants are licensed: MPL licensed in the United States as part of the AS04 adjuvant formulation [23], and *Bordetella pertussis* component vaccine adjuvant [24] that is used in several licensed vaccines. Microorganism-derived adjuvants cholera toxin and MPL are used in 17 and 10 vaccines, respectively. Many microorganism-derived adjuvants are based on microbial nucleic acids. For example, CpG DNA is the unmethylated CpG dinucleotides in what is called motifs, which are abundant in microbial DNA, but not in the DNA of vertebrates [25]. CpG DNA is immunostimulants that have the ability to activate Th1 immunity [26].

All but one microorganism-derived adjuvants act as immunostimulants as their mechanism of action. Adjuvants derived from the toxins of bacteria are used to stimulate mucosal immunity, which is important for protecting against many pathogens that are contracted mucosally [27]. The route of administration is able to reveal what type of immunity the adjuvant can stimulate, with eight microorganism-derived adjuvants being mucosally administered (generally intranasally). These are adjuvants which can help to stimulate mucosal immunity which is often critical in inducing protection from pathogens that are contracted mucosally. Six of the adjuvants are administered both parenterally and mucosally, and five are parenterally administered (Table 4).

3.2.4. Other Vaccine Adjuvants. The other six adjuvant types are synthetic adjuvants, cytokine adjuvants, particulate antigen delivery systems, carbohydrate vaccine adjuvants, tensoactive compounds, and combination vaccine adjuvants (Table 1).

Synthetic adjuvants are synthetically made compounds that mostly are utilized as immunostimulants and occasionally act as carriers. There are 28 synthetic adjuvants in Vaxjo. Some are derived to mimic bacterial components (Table 1). For example, muramyl dipeptide (MDP) is formulated to imitate a component found in the cell walls of mycobacteria used in Freund's complete adjuvant [28]. In addition, many synthetic MDP analogs are used as vaccine adjuvants. Administration routes are diverse, as there are parenterally, mucosally, and also topically administered synthetic vaccine adjuvants.

Cytokines, signaling molecules of the immune system, are also used as adjuvants to elicit a specific immune

Table 4: Microorganism-derived adjuvants.

Adjuvant name	Number of Vaccines	Mechanism	Route	Licensed
Cholera toxin	17	Immunostimulant	Mucosal	No
CpG DNA vaccine adjuvant	12	Immunostimulant	Either *	No
MPL adjuvant	10	Immunostimulant	Either	As part of AS04
Cholera toxin B subunit	6	Immunostimulant	Mucosal	No
LTR192G vaccine adjuvant	6	Immunostimulant	Mucosal	No
Bordetella pertussis component vaccine adjuvant	3	Immunostimulant	Parenteral	Yes
E. coli heat-labile toxin, LT	3	Immunostimulant	Mucosal	No
CTA1-DD gene fusion protein	2	Immunostimulant	Either	No
Etx B subunit adjuvant	2	Immunostimulant	Either	No
Killed *Corynebacterium parvum* vaccine adjuvant	2	Immunostimulant	Parenteral	No
Lipopolysaccharide vaccine adjuvant	2	Immunostimulant	Either	No
LTK63 vaccine mutant adjuvant	2	Immunostimulant	Mucosal	No
Corynebacterium-derived P40 vaccine adjuvant	1	Immunostimulant	Parenteral	No
Flagellin vaccine adjuvant	1	Immunostimulant	Either	No
LTK72 vaccine adjuvant	1	Immunostimulant	Mucosal	No
MPL-SE vaccine adjuvant	1	Immunostimulant	Parenteral	No
Non-toxic mutant E112K of Cholera Toxin mCT-E112K	1	Immunostimulant	Mucosal	No
Ty particles vaccine adjuvant	1	Vehicle	Parenteral	No

Note: *either means either mucosal or parenteral route.

response [23]. There are nine cytokines as adjuvants in Vaxjo (Table 1). For example, granulocyte-macrophage colony-stimulating factor (GM-CSF), a cytokine that activates mature granulocytes and macrophages, is being used as an adjuvant for Hepatitis B in immunocompromised patients [29]. The GM-CSF adjuvant is also used in vaccines for HIV and cancer [30, 31]. Currently, none of cytokine-based adjuvants are licensed.

Particulate antigen delivery systems are adjuvants that have a depot effect for the antigen and include liposomes, polymeric microspheres, virus-like particles, and immunostimulating complexes (ISCOMs) [13]. There are nine particulate antigen delivery systems in Vaxjo. These adjuvants act as vehicles for the antigen, while often also containing immunostimulating compounds. They are parenteral adjuvants. Liposome-based adjuvants consist of spheres formed by lipid layers that can encapsulate antigens and thus act both as a vehicle and an immunostimulant [1]. Six liposome-based vaccine adjuvants are listed in Vaxjo. MTP-PE liposomes are liposomes that contain a synthetic analog of MDP, MTP-PE. It is used in vaccines for hepatitis B and herpes simplex virus [32, 33].

Carbohydrate adjuvants are adjuvants composed of complex carbohydrates of natural origin that stimulate the immune system. The main sources of these carbohydrates are plants and fungi [1]. For example, gamma inulin is a carbohydrate derived from the root of plants of the *Compositae* family. Gamma inulin is an activator of humoral and cellular immunity as well as the alternate complement pathway [1]. Two carbohydrate adjuvants are curated in Vaxjo (Table 1). They are generally parenterally administered and are immunostimulating adjuvants.

Tensoactive compounds are surfactants or active surface agents. They are immunostimulating adjuvants generally derived from plants, namely, *Quillaja saponaria.* There are five tensoactive adjuvants in Vaxjo (Table 1). Most saponins are considered too toxic for human use, although Quil A has been used in animals [1]. The saponins act as a vehicle/immunostimulant and are parenterally administered.

Combination vaccine adjuvants include two or more different adjuvants that are used in combination. Examples of combination adjuvants include adjuvants such as AS04 and Algammulin. There are six such adjuvants in Vaxjo (Table 1). These adjuvants combine different mechanisms of action from two or more adjuvants [13]. For example, AS04, a licensed vaccine in the United States, is a combination of aluminum and MPL [34]. Alum and MPL complement one another: Alum provides signals required for generating long-lived memory cells, whereas MPL enhances the differentiation of cytotoxic T lymphocytes (CTL) [35].

3.3. Analysis of Vaccine Adjuvants Based on the Pathogen Types. Table 5 displays adjuvanted vaccines by pathogen/disease types and vaccine types. Of the pathogens/diseases in VIOLIN, 46.2% have vaccines that use adjuvants. Gram-positive pathogens and parasites are associated with a high percentage of adjuvanted vaccines, with 79% and 71% of species

Table 5: Vaccines with adjuvants by vaccine-targeted pathogens or diseases.

Pathogen or disease	Number of pathogen species	Subunit vaccines	Toxoid vaccines	DNA vaccines	Conjugate vaccines	Live vaccines	Killed vaccines	RCV vaccine**	VDP***	Total
Gram+ bacteria	11/14*	39	26	2	6	0	4	1	18	96
Gram− bacteria	22/48	96	6	2	7	0	2	5	13	130
Viruses	27/87	43	0	5	0	2	19	1	13	83
Fungi	1/1	3	0	0	0	0	0	0	0	3
Parasite	15/21	50	0	1	0	1	1	2	0	55
Cancer	—	11	0	1	0	1	0	0	0	13
Allergy	—	1	0	1	0	0	0	0	0	2
Total	80/173	245	32	14	13	4	26	9	44	384

* 11/14 means 11 out of 14 spp. that have adjuvanted vaccines. Other numbers in this column use the same representation.
**RCV stands for recombinant vector vaccine.
*** VDP stands for vaccine made with different preparation methods, including (1) vaccine containing subunit component and inactivated whole organism, (2) vaccine containing DNA vaccine for priming and subunit vaccine for boosting, and (3) vaccine containing DNA vaccine for priming and Ad vector vaccine for boosting.

containing adjuvanted vaccines, respectively. Meanwhile, 45.8% of Gram-negative pathogen vaccines use adjuvants. Of 1,982 viral vaccines in VIOLIN, only 4% are adjuvanted. For the cancer and allergy vaccines in VIOLIN, 20.83% use adjuvants. 13% of all the vaccines in VIOLIN are adjuvanted.

To develop vaccines for a specific pathogen, various strategies, including different vaccine adjuvants, may have been used. The choice of using different vaccine adjuvants may be dependent on the type of protective immunity required for the specific pathogen. As an example of illustrating these points, here we summarize and compare all vaccine adjuvants used in development of *Brucella* vaccines. *Brucella*, a Gram-negative intracellular pathogen that causes brucellosis in humans, is a disease for which there is no licensed human vaccine. Although humoral immune response against *Brucella* lipopolysaccharides (LPS) provides partial protection, the cell-mediated immunity plays a major role in protection against virulent *Brucella* challenge [36, 37]. In VIOLIN, 45 *Brucella* vaccines are curated. Of these 45 *Brucella* vaccines, nine use adjuvants (Table 6). Among these nine vaccines, six are subunit vaccines [38–42]. This phenomenon follows the trend of subunit vaccines being the majority of vaccines that use adjuvants. Among three of these six subunit vaccines that use Freund's adjuvants, two of them use complete Freund's adjuvant for the primary immunization and incomplete Freund's adjuvant for the boost vaccination [40, 41]. Three other vaccines are not categorized as subunit vaccines [43–45]. Among them is a vaccine that uses a DNA vaccine for priming and uses a recombinant protein adjuvanted by incomplete Freund's adjuvant for boosting [45]. The NPAP *Brucella* vaccine is a killed vaccine that uses Freund's incomplete adjuvant [43]. The recombinant *O. anthropi* expressing *Brucella* Cu/Zn SOD is live vaccine that uses with CpG DNA adjuvant [44]. The CpG DNA adjuvant is able to bias the immune response to the *Brucella* antigen towards a cell-mediated immunity. The recombinant *O. anthropi* expressing *Brucella* Cu/Zn SOD alone without the CpG DNA adjuvant is not able to induce protection, indicating the importance of the cell-mediated immunity in the protection induction [44].

Although most subunit vaccines use adjuvants, some subunit vaccines do not use adjuvants. For example, two of these *Brucella* subunit vaccines contain *Brucella* lipopolysaccharide (LPS) as a primary antigen that can also perform as an adjuvant-like function by inducing cell-mediated immunity [46, 47]. The recombinant chimera vaccine BLSOmp31 includes a known *Brucella* Omp31-derived protective epitope fused to *Brucella* lumazine synthase (BLS) [48]. The BLS is highly immunogenic and functions as a scaffold protein and a carrier for the foreign peptide [49].

3.4. Analysis of Vaccine Adjuvants Based on Vaccine Types. The majority of adjuvanted vaccines, with 245 out of 384 vaccines (63.8%), are subunit vaccines. Other types of adjuvanted vaccines stored in VIOLIN include toxoid vaccines (8%), killed vaccines (7%), DNA vaccines (4%), and conjugate vaccines (3%). Forty-three vaccines in VIOLIN contain more than one adjuvant (http://www.violinet.org/vaxjo/stat2.php). In many cases, a primary vaccination uses one adjuvant, and then a boost immunization uses another adjuvant. Alternatively, one vaccine includes more than one adjuvant for one vaccination.

A subunit vaccine is composed of a purified protein(s) or other antigenic determinant(s) from a disease-causing organism. A subunit vaccine does not include pathogen nucleic acids. The subunits have less risk of causing adverse reactions. Subunit vaccines usually consist of specific proteins targeted to generate protection; however, they are rarely immunogenic on their own to trigger a response that will generate protection [50]. The very nature of subunit vaccines generates a need for adjuvantation. The vast majority of vaccines using adjuvants are subunit vaccines, representing 63.8% of the adjuvanted vaccines in VIOLIN (Table 5). In Vaxjo, 53 of the vaccine adjuvants are used in subunit vaccines. Most of these adjuvants have not frequently been used and occur only in one or two vaccines. A few are used more frequently, such as aluminum hydroxide, aluminum phosphate, Alhydrogel, Freund's complete and incomplete adjuvants, and cholera toxin.

Table 6: *Brucella* vaccines with adjuvants in VIOLIN.

Vaccine name	Vaccine type	Adjuvant name	Reference
B. melitensis DNA vaccine encoding Omp31 boosted with Omp31	DNA vaccine with recombinant protein	Incomplete Freund's adjuvant	[45]
B. melitensis P39 protein vaccine	Subunit vaccine	CpG DNA vaccine adjuvant	[38]
Brucella ovis microparticle subunit vaccine	Subunit vaccine	Lipopolysaccharide adjuvant	[39]
Brucella recombinant SurA protein vaccine	Subunit vaccine	Incomplete Freund's adjuvant Complete Freund's adjuvant	[40]
E. coli Escheriosome-mediated cytosolic delivery of recombinant *Brucella* rL7/L12 protein	Subunit vaccine	Complete Freund's adjuvant	[41]
NPAP *Brucella* vaccine	Inactivated or "killed" vaccine	Incomplete Freund's adjuvant	[43]
Porin and S-LPS extracted from virulent *Brucella abortus* 2308	Subunit vaccine	MPL vaccine adjuvant	[42]
Recombinant *Brucella* DnaK protein vaccine	Subunit vaccine	Incomplete Freund's adjuvant Complete Freund's adjuvant	[40]
Recombinant *O. anthropi* 49237SOD	Recombinant vector vaccine	CpG DNA vaccine adjuvant	[44]

The most frequently adjuvanted killed vaccines, with 19 out of 26, are viral vaccines (Table 5). Twelve adjuvants have been used in killed vaccines, with the most frequently used being aluminum hydroxide. The list of the US licensed vaccines that contain aluminum adjuvants include Anthrax vaccine, Hepatitis A and B vaccines, Rabies vaccine, Human papillomavirus vaccine, Pneumococcal conjugate vaccine, DTP (diphtheria-tetanus-pertussis vaccine) [19]. However, licensed human inactivated Polio Virus (IPV) vaccine, measles, mumps and rubella vaccine (MMR), varicella vaccine, and influenza vaccines do not contain any aluminum salt vaccine adjuvant [50]. Some of the other adjuvants used in killed vaccines in research are QuilA, aluminum phosphate, AS03, AS04, AF03, and Ribi vaccine adjuvant.

A toxoid vaccine consists of a toxoid (also called inactivated toxin), which is a processed toxin that has been treated by chemical means, heat, or irradiation and is no longer capable of causing disease. Toxoid vaccines, which are similar to subunit vaccines, are also usually adjuvanted, typically with aluminum. There are nine adjuvants associated with 32 toxoid vaccines (Table 5), which include aluminum adjuvants, squalene, and nanoemulsion vaccine adjuvant. The most frequently used is aluminum phosphate, which is used in nine toxoid vaccines.

A conjugate vaccine is vaccine that conjugates/links antigens to the molecules that form the outer coat of disease-causing bacteria to promote an immune response. Conjugate vaccines are not often adjuvanted, as they are usually immunogenic enough to not need an adjuvant. However, there are seven adjuvants in Vaxjo that are used in 13 conjugate vaccines (Table 5), including aluminum hydroxide, DDA, Ribi vaccine adjuvant, and MPL.

Live vaccines generally do not need adjuvants as they are rather immunogenic on their own. However, there are five vaccine adjuvants in Vaxjo that are used in four live attenuated vaccines (Table 5). These include aluminum hydroxide, Freund's complete and incomplete adjuvants, DHEA vaccine adjuvant, and Arlacel A. These three vaccines are for protection against Venezuelan equine encephalitis virus (VEE), cancer, smallpox, and *E. maxima*. For these vaccines, the adjuvant was given prior to immunization with the live attenuated vaccine.

Similar to live attenuated vaccines, DNA vaccines are not often adjuvanted. As for DNA vaccines, there are also eight adjuvants used that are in Vaxjo. There are 14 DNA vaccines that use adjuvants use GM-CSF, Resiquimod, and DDA among others (Table 5).

3.5. Ontology-Based Vaccine Adjuvant Representation for Exchange, Transfer, and Download of Vaccine Adjuvant Data. The basic information of vaccine adjuvants in Vaxjo is now stored in the Vaccine Ontology (VO; http://www.violinet.org/vaccineontology/) [17]. A biomedical ontology is a set of terms and relations that represent entities in a biomedical domain and how they relate to each other. Ontology terms are associated with documentation and definitions, which are, ideally, expressed in formal logic in order to support automated reasoning [51]. VO is a community-based ontology in the vaccine domain. It is developed based on the Web Ontology Language (OWL) format (http://www.w3.org/TR/owl-ref/), which can be processed by many existing software programs and used for Semantic Web applications. The storage of vaccine adjuvants in VO allows development of new software programs to integrate the vaccine adjuvant data with other biomedical data and support further bioinformatics analyses.

Figure 3 provides an example of how a VO vaccine adjuvant term "aluminum vaccine adjuvant" is shown in VO. The top level terms "entity → continuant → independent continuant → material entity" come from the Basic Formal Ontology (BFO: http://www.ifomis.org/bfo/), which is a top

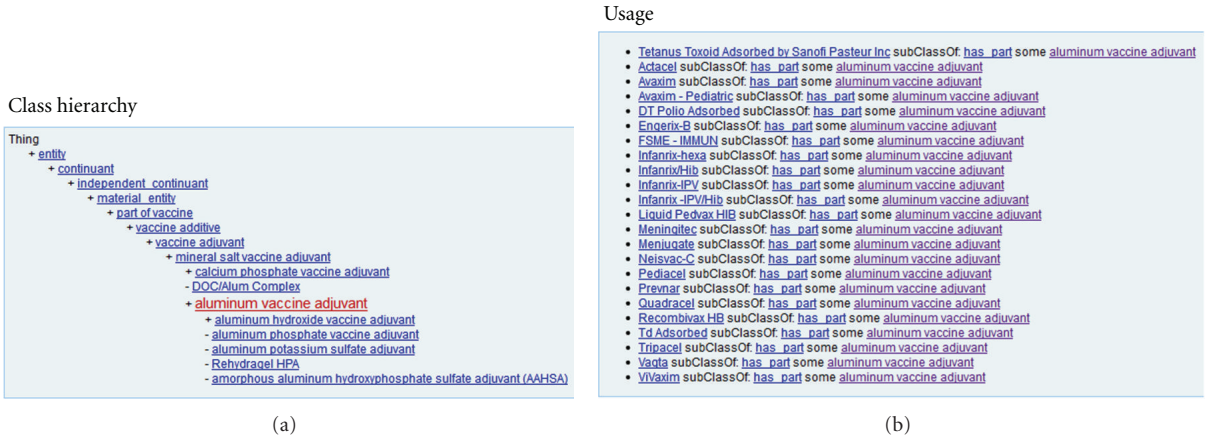

Figure 3: Modeling and application of vaccine adjuvants in the Vaccine Ontology (VO). (a) The hierarchical structure of vaccine adjuvants in VO. (b) All the vaccines that use this adjuvant. The usage information is obtained by a SPARQL query based on the logical definitions of vaccines. The website that contains this information is http://purl.obolibrary.org/obo/VO_0000884.

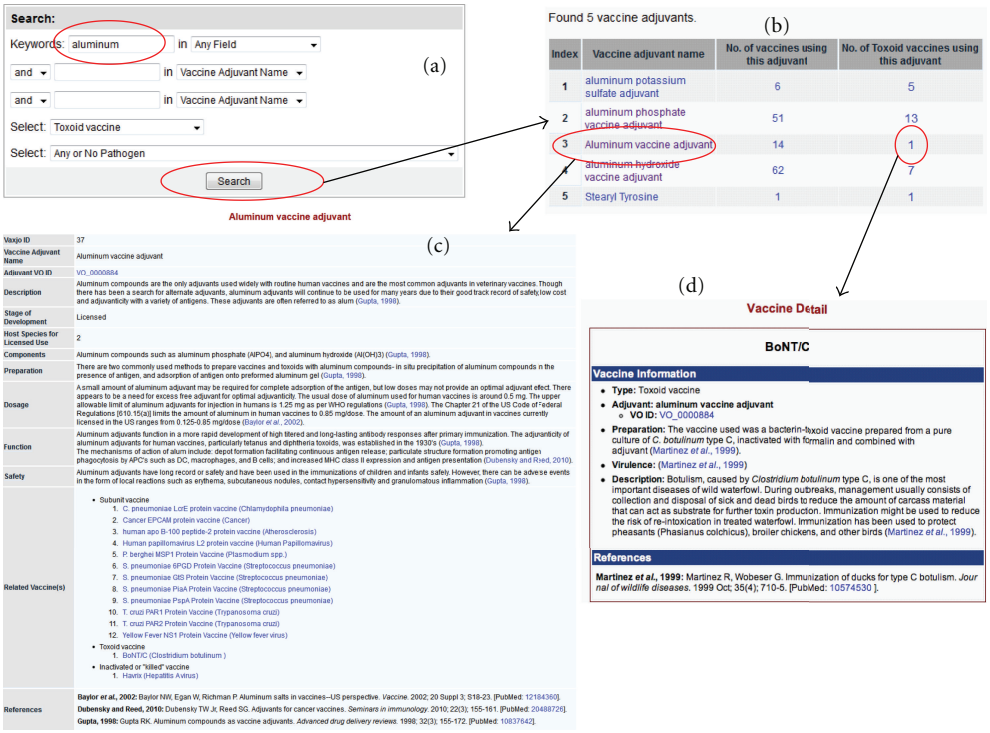

Figure 4: An example of searching a vaccine adjuvant and its related information in Vaxjo. (a) The keyword "aluminum" is queried in Vaxjo. (b) The adjuvants resulting from the Vaxjo keyword search. (c) The Vaxjo page obtained by clicking on "Aluminum vaccine adjuvant" in (b). The Vaxjo page for the adjuvant has information about the adjuvant (alternate names, components, function, etc.) and lists vaccines in which the adjuvant is used, as well as references. (d) The vaccine page obtained by clicking on the vaccine number shown in (b). This web page shows the details about this vaccine.

ontology that covers the universal entities. A continuant (e.g., a person or a quality) is an entity that exists in full at any time. An independent continuant (e.g., a person which is a material entity, but not a quality) is a continuant that bears its quality and is independent of other entities. All the other terms in the hierarchy shown in Figure 3(a) are VO-specific terms. As a part of a vaccine, a vaccine additive (e.g., preservative and adjuvant) is a material entity that is added to the immunogen of a vaccine. An aluminum vaccine adjuvant is a mineral salt vaccine adjuvant, a common vaccine adjuvant type (Figure 3(a)). Figure 3(b) indicates all vaccines that use this aluminum vaccine adjuvant (including all it

child terms). One advantage of this OWL format is that it can be parsed and read by many computer programs. The machine readable feature supports automated reasoning.

3.6. Vaxjo Data Query and Display. The manually curated and precomputed Vaxjo data can be efficiently queried and visualized as demonstrated in Figure 4. The vaccine adjuvant can be queried by specifying one or multiple criteria: (1) a characteristic of a vaccine adjuvant, including name, alternative name, adjuvant VO ID, description, components, and storage, (2) vaccine type, and (3) pathogen (Figure 4(a)). All query hits are first displayed on a web table containing basic adjuvant information (Figure 4(b)). Individual vaccine adjuvant can further be selected by a user to show more detailed information (Figure 4(c)). The associated vaccines are viewed by clicking on the number of associated vaccines (Figure 4(d)).

4. Discussion

Vaxjo contains manually curated vaccine adjuvants that have been used in a wide array of vaccines for a large variety of infectious diseases, as well as diseases such as cancer and allergies. To the best of our knowledge, Vaxjo is the first web-based, publically available database and analysis system that targets for the curation and analysis of vaccine adjuvants. There is a database of adjuvants/stimulants that is part of a nonhuman primate HIV/SIV vaccine trials database (http://www.hiv.lanl.gov/content/vaccine/adjuvants-stimulants.html). However, this database focuses on adjuvants used in HIV/SIV vaccine trials, which is a much narrower focus than Vaxjo. While many adjuvant review papers also exist [1, 2, 12, 13, 19, 50, 52], the information of vaccine adjuvants introduced in these review papers cannot be queried online and updated. The original VIOLIN database also includes many vaccines using different adjuvants. However, the general VIOLIN database does not include classification and details about each vaccine adjuvant. The Vaxjo database and query system is unique in that Vaxjo integrates the information of individual adjuvants and their uses in development of different vaccines stored in the general VIOLIN database. Therefore, Vaxjo is not just one simple extension of the VIOLIN vaccine database. The Vaxjo database is a new and relatively independent program.

A few challenges have been identified throughout the vaccine adjuvant curation process. One challenge is that many adjuvants are proprietary, and therefore there is little information available about them. There are many licensed animal vaccines that reside in VIOLIN, that lack adjuvant information. Because there is little information available on these vaccines, it is possible that some of these vaccines are in fact adjuvanted.

The Vaxjo database and analysis system can be used to facilitate rational vaccine design. Many diseases are complex in their interactions with the host immune system, thus making vaccine design difficult. To induce a protective immunity against a specific disease, an appropriate vaccine adjuvant is often required. Due to the large number of adjuvants in the market, it is often difficult to decide which vaccine adjuvant to use. The Vaxjo database provides details for each adjuvant and how they have been used in development of effective vaccines against different types of diseases. Therefore, an efficient query, comparison, and analysis of the data stored in Vaxjo allows a researcher to identify a small tailored list of vaccine adjuvants that can be used for enhancing induction of desired immune responses and having the highest possibility of success.

As a central and vital source of vaccine adjuvants, Vaxjo is a timely repository and is expected to have a significant impact for vaccine research and development.

Acknowledgment

This research was supported by NIH-NIAID Grant R01AI081062.

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