Novel Adjuvants for Vaccines

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Abstract: The review covers basic recent discoveries on the mechanism of action of adjuvants, which have been developed in the last decade. In particular, it focuses on the activation of TOLL like receptors and subsequent release of pro-inflammatory lymphokines (innate immunity), which, in turn, are thought to activate antigen specific responses (adaptive immunity). Given the number of novel adjuvants available to researchers, we limited the scope of the review to those adjuvants which are routinely used in our laboratories and of which we have first hand information.

Keywords: Adjuvant, vaccine, T cell, antibody, delivery, TLR.

1. INTRODUCTION

Adjuvants are substances that, when used in combination with vaccine antigens, induce a stronger and more efficacious response to the vaccine as compared to that induced by the vaccine alone. Most vaccines currently used, especially those consisting of inactivated (killed) microorganisms and those containing purified molecules, are given together with adjuvants. Despite the major efforts made in the past decades to develop new vaccine adjuvants [1] the first adjuvants ever reported in the scientific literature, i.e. aluminium salts, still remain the standard adjuvants approved for use in human beings.

The development of aluminium salts as the first adjuvants and the development of the first subunit vaccines are strictly linked together. An ideal subunit vaccine contains pathogen antigen(s) which has been shown or inferred to induce a protective immune response. Thus, historically, bacterial toxins in their inactivated forms remain the first subunit vaccines developed. This was made possible by the demonstration of the anti-tetanus immunity conferred to rabbits by immunization with attenuated tetanus toxin [2], and by the demonstration that formalin treatment fully inactivated the diphtheria toxin and that guinea pigs immunized with the formalin-treated toxin were protected against a lethal challenge [3,4]. The first attempts to enhance the immunogenicity of these toxoids in animals were reported by Ramon [5], who observed that the highest antibody titers were obtained in animals in which local inflammatory reactions were induced by the concomitant injection of other substances, such as sterilized tapioca, aluminium salts, lanolin, tannin, kaolin, etc. The use of aluminium hydroxide as an adjuvant to increase the immunogenicity of the diphtheria toxoid was definitely introduced the following year by Glenny et al. 1926 [6].

Modern approaches towards the development of new vaccines preferentially foresee the use of purified components of the microorganisms, in general, as recombinant proteins or synthetic peptides. In the case the pathogen antigen(s) is represented by bacterial poly- or oligosaccharides, a suitable carrier such as a conjugated protein must be envisaged. These highly purified proteins are likely to be less immunogenic than the traditional toxoids and inactivated vaccines, which contain impurities that are often not well characterized at the molecular level. In addition, optimal incorporation, either through absorption or entrapment, of the immunogen on/in alum salt precipitate is required for full adjuvant activity of the aluminium salts. Thus, the need to obtain protective immune responses for new generation vaccines required new advances in the understanding of the mechanism of the adjuvant effect and a larger choice of new powerful adjuvants. For new generation vaccines, adjuvants that exert stronger adjuvancy than aluminium salts are required to induce appropriate effective protective immunity.

It is recognized that the correct choice of the vaccine molecule and the appropriate adjuvant system would be capable to induce the most appropriate immune response to confer protection. We had to wait until the second half of 1990’s to see new vaccine adjuvants being approved for use in humans. Several reasons may have limited the adoption of new vaccine adjuvants [7], among which are safety issues, appropriateness of a given adjuvant for the particular vaccine in terms of physical interaction, quality of the effector immune response to be induced and route of immunization. At the present, through a better understanding of the basic mechanisms of adjuvanticity and the discovery of molecules that activate these mechanisms, there is hope for the development of new efficacious, tailor-made formulations.

2. ADJUVANTS

2.1. General Considerations on Mechanisms of Action and Routes of Delivery

Despite vaccine adjuvants have been in use for more than seventy years, very little is known on the exact mechanisms behind their biological effect. The depot effect postulated for aluminium salts and for other adjuvants cannot by itself
explain all the immunological phenomena that are triggered by vaccine adjuvants and mediate the activation and recruitment of professional antigen-presenting cells (APC) which, in turn, induce activation and expansion of antigen-specific T and then of B cell populations. The depot effect of aluminium salts, resulting in a reduced rate of antigen clearance from the site of injection, was first suggested by Gleny et al. [8]. However, a large series of experimental data has clearly shown that a slow release of the vaccine over time is very unlikely to contribute to the adjuvanticity of aluminium compounds [9]. Many adjuvants, including aluminium salts, have been postulated to exert their adjuvanticity through their ability to activate APC and to increase the expression of major histocompatibility complex (MHC) class II molecules and of several co-stimulatory molecules. However, the exact mechanisms leading to these phenomena are still unclear. A possible role of aluminium salts is described in a very recent publication in which activation of a alum-induced myeloid cell population is postulated [10].

During the past few years, research on innate immunity has experienced a large expansion, and new concepts related to the adjuvant effect have been put forward and validated [11]. This has led to the identification of several molecules and their relevant ligands as important elements in the first line of defense against invading microorganisms. These findings are turning out to be instrumental in understanding the mechanisms of adjuvanticity of several substances, mainly those derived from bacterial components. The family of the Toll-like receptors (TLR), firstly described in Drosophila, is emerging as a key player in the delivery of initial signals not only by invading pathogens, but also by some of their components, which can eventually be used as vaccine adjuvants.

Activation of cells by bacterial lipopolysaccharide (LPS) requires the presence on APC of an intact TLR4; this will induce the expression of inflammatory cytokines and of costimulatory molecules [12]. In addition, TLR4 is also implicated in the recognition of the 60 kDa heat shock protein (hsp60) [13] and lipoteichoic acid [14], which were already known for their adjuvant activity [1].

By forming heterodimers with TLR1 and TLR6, TLR2 recognizes several microbial components, such as peptidoglycan, bacterial lipoproteins, and zymosan [15,16]. It is likely that the strong adjuvant activity of complete Freund’s adjuvant, which contains mycobacteria, and of monophosphoryl lipid A (MPL), derived from the bacterial cell wall, may be at least partly explained by the interaction with TLR2.

Finally, the strong immuno-stimulatory effect of unmethylated CpG oligodeoxynucleotides, which leads to strong induction of Th1-type response, has been shown to be mediated by a specific recognition of TLR9 by these motifs [17]. This leads to activation of macrophages and dendritic cells (DC), up-regulation of MHC class I molecules and of co-stimulatory molecules, and to the production of pro-inflammatory (Th1-type) cytokines.

Several adjuvants, however, do not contain any microbial components and, thus, there is no indication that their mechanism(s) of action is mediated by interactions with TLR. For example, it is very unlikely that aluminium salts exert their adjuvanticity through a TLR, since aluminium hydroxide exerts good adjuvanticity in mice genetically knocked-out at the level of MyD-88, an adaptor molecule in the TLR-signaling pathway [18]. Similarly, biodegradable microcapsules made of PLGAand or PLA do not seem to activate any of the TOLL-like molecules (unpublished results).

Another point deserves comment. It is now very well known that adjuvants can profoundly affect the polarization of the T-cell response (and thereby of the effector – and possibly protective - immune response) induced by vaccines. It is known that aluminium adjuvants can induce in animals and in humans the production of antigen-specific IgE antibody [19,20]. This is very likely mediated by the high propensity of aluminium salts to polarize the CD4 \(^+\) T cell response towards a Th2-type response, through an increased expression of IL-4 [10]. At the other extreme, there is the example of the unmethylated CpG oligodeoxynucleotides, which lead to an exquisite, strongly polarized CD4 \(^+\) Th1-type response. In addition, activation of antigen-specific cytolytic CD8 \(^+\) cell may require the use of other adjuvants and/or delivery systems, such as bacterial or viral delivery systems, immunization with naked DNA, lipiddization of antigens, encapsulation or simple adsorption of antigens into or onto microparticles.

Finally, because of their intrinsic anatomical characteristics and being the portal of entry of most pathogens, mucosal surfaces represent ideal sites for vaccine delivery. Live-attenuated vaccines (e.g. polio and Salmonella vaccines) are given orally. However, non-replicating, purified antigens are poorly immunogenic when delivered mucosally and may even induce a state of tolerance (refs). The best mucosal immunogens are those with an inherent ability to attach to epithelial cells, e.g. the Escherichia coli heat-labile enterotoxin (LT), the cholera toxin (CT), bacterial fimbriae, some lectins, etc. [21]. The existing adjuvants and the vast majority of those under investigations are primarily intended for parenteral administration. It is thus clear that strong and safe adjuvants specifically designed for delivery of vaccines at the mucosal surface are highly desirable. A wealth of exciting research is currently being devoted to this issue, and it will be briefly summarized in this chapter.

2.2 New Vaccine Adjuvants

Vaccine adjuvants can be evaluated as such only when they are associated with a vaccine. The vaccine to be adjuvanted is, in general, of proteic origin through purification of natural, recombinant or synthetic products. This holds true also for carrier-hapten systems of various nature. Consequently, in the following sections, we will discuss the properties and use of adjuvants in combination with proteic vaccines.

In considering the development of vaccines for the entire world and major diseases, one has to focus attention to a number of specifications, besides potency and safety. For example, feasibility in obtaining large quantity of the product at reasonable cost, the easiness of the preparation and usage and stability of the formulation in case of failure to maintain...
the cold chain conditions are extremely important considerations in vaccine/adjuvant development. The ideal formulation would be a product that is stable above room temperature, has a long shelf-life and does not require needles for administration. As today, not a single product has met all of these desired characteristics, even though some of them present promising properties.

In the following, we will group the various adjuvant formulations under investigation according to their physical nature: 1) water-soluble adjuvants, 2) oil-in-water or water-in-oil emulsions, 3) adjuvants that entrap and/or adsorb the antigen and 4) particulates for antigen delivery (i.e., virosomes, liposomes, styrene beads etc., not discussed here).

Given the present large number of available adjuvants, it may at times be difficult to choose the most appropriate one. One must carefully consider the patent status and availability of the adjuvant(s) in addition to the desired immunological and physical properties of the final formulation. Very importantly, a selected adjuvant should be used throughout the pre-clinical and clinical studies. From our experience, it is advisable to perform a pilot study, in which several adjuvants can be compared, and then select one or two for further studies. The caveat is that results obtained in animal studies may not be reproduced in clinical trials. In addition, studies using alum as baseline should also been considered since, at the present, regulatory guidelines require a comparative study in humans in order to assess the superior effect of a chosen adjuvant over alum. In the following sections, we will restrict our discussion to adjuvants for which we have first-hand information and experience. We would like to point out the pros and cons of each adjuvant tested in our laboratory keeping in mind that our experience might be limited to few antigens and, thus, may vary if different systems are used. Readers interested in more detailed, technical information on specific adjuvants not covered here are referred to the literature published in the past few years [22-24].

2.2.1 Water-Soluble Adjuvants

The few water-soluble adjuvants we have been using are OM-174 and QS-21 analogues. Both adjuvants are highly suitable for vaccines development since they are soluble in aqueous buffers. Vaccine formulations are readily obtained by mixing appropriate quantities of both the actual antigen and the adjuvant. The mixture is generally stable for hours, if not days.

OM-174 is a soluble derivative of mono-phosphoryl lipid A (MPL) obtained through chemical synthesis [25]. It is a proprietary product of OM-Pharma (Meyrin, Switzerland) and available through collaboration with OM-Pharma. It induces dendritic cell maturation probably mediated through TLR-4 [26,27]. In our laboratory, OM-174 has been extensively used in several strains of mice and rabbits always giving satisfactory results. Our model antigens are long or short synthetic peptides derived from malaria proteins of Plasmodium falciparum or Plasmodium berghei. All arms of the immune systems were activated including CD8+ T cells [28]. The antibody response did not differ from that elicited by other adjuvants like QS-21, Montanide ISA 720 and 51 or Incomplete Freund’s adjuvant (G. Corradin and collaborators, unpublished results). One difference observed with respect to other adjuvants was the slower rate of increase in antibody titers upon successive boostings, a difference that vanished after the second or third booster injection. Full protection against a challenge with live sporozoites was obtained in mice after two injections of OM-174 and the C-terminal fragment 242-310 derived from the circumsporozoite protein (CSP) of P. berghei. Given the high solubility of the product, we speculated that it may be useful to co-adsorb OM-174 and the antigen on alum to enhance the association of the CSP antigen and the adjuvant. This comparative study did, however, not reveal any major difference in the overall response and protection (ref). Clinical studies of this adjuvant alone or together with a malaria synthetic subunit vaccine indicated excellent safety of the product R. Audran and F. Spertini, personal communication). It is our opinion that this adjuvant is ideal for parenteral inoculation of vaccines either alone or in conjunction with other adjuvants (e.g. alum). Other derivatives of OM-174 were developed by OM-Pharma, all retaining similar properties of the original product.

QS-21 is an acetylated 3,28-o-bisdesmodic triterpene saponin (190 MW) derived from the bark of the South American tree Quillaja saponaria Molina [29], and is soluble in aqueous buffer. Availability is through collaboration with Antigenics, Boston, MA. Adjuvant activity is not mediated by TLR-2 and –4 [27]. Tested in different animal models and also in several human trials with different antigenic components (e.g., from HIV-1, influenza, HSV, HBV, malaria, cancer), QS-21 appears to augment both Th1- and Th2-type responses [30] and to favour the in vivo priming of antigen-specific CD8+ cytotoxic cells [31]. In HIV clinical trials comparing different adjuvants, QS-21 was associated with more severe local reactions [32]; it appears, however, that local biocompatibility ameliorates by decreasing the amount of adjuvant used.

When we used QS-21 together with P. berghei CSP C-terminal fragment 242-310, the vaccine has given a remarkable response in terms of activation of CD8+ T cells specific for the MHC-K restricted epitope 245-253 in liver and spleen. In fact, the amount of tetramer positive CD8+ T cells in the two organs was about 7 and 4 %, respectively after two injections. With all other adjuvants we have tested, this specific CD8+ T cell response was 5-10 times lower. The corresponding degree of protection obtained in the experimental challenge model with live sporozoites reached a level of 60%. While this level is satisfactory, one would have expected to reach full protection as with other adjuvants (Meraldi et al., manuscript submitted). Since the protection experiments were not run in parallel and one cannot really strictly compare the results due to possible variations of health and housing status of mice in the different experiments, it would be important at this point to run a adjuvant comparative challenge experiment to determine whether or not a stronger CD8+ T cell response is less efficient than a weaker one in conferring protection. If confirmed, this would have profound consequences in vaccine design at least for pre-erythrocytic malaria vaccine formulations.
QS-21 has been extensively used in conjunction with MPL in the development of a pre-erythrocytic malaria vaccine in phase II and III trials giving satisfactory results [33,34].

A special example of soluble adjuvants is the mucosal adjuvants derived from bacterial toxins. The ADP-ribosylating toxins E. coli enterotoxin (LT) and the colera toxin (CT) remain the strongest mucosal adjuvants known so far. Their toxicity, however, seriously limits their use in humans since very limited amounts cause diarrhoea in the recipients [35]. The most successful approach to develop non-toxic derivatives of the toxins still retaining their strong mucosal adjuvanticity has been to produce mutants by site-directed mutagenesis [36,37]. Some mutants at the active enzymatic site, such as the ones at position 63 in the A subunit (e.g. LTK63 and CTK63 [S → K substitution]) have totally lost their enzymatic activity and their toxic properties in vitro and in vivo, even at doses up to 1 mg. Other mutants, such as the LTR72 (A → R substitution), retain some residual enzymatic and toxic activity, although at levels several orders of magnitude lower than that observed with the wild type toxin [38]. Mutants have also been originated to make the toxin resistant to the proteolytic cleavage of the A subunit.

All these mutants (and others not reported here because of space limitation) behave as strong mucosal adjuvants in mice and in other animal models, when co-administered together with recombinant proteins, synthetic peptides, and other vaccine constructs, and to favor protective immunity in appropriate models of challenge[37]. Interestingly, LTK63 and LTR72 mutants exhibited a strong adjuvanticity also when delivered systemically [39] and trans-dermally [40]. The mucosal adjuvanticity of these mutants was potentiated when the vaccine and the adjuvant were delivered intranasally together with nanoparticles and other delivery systems (e.g. chitosan derivatives) favoring the uptake of the vaccine at the mucosal level [41-43]. Furthermore, not only these mutants primed CD4+ T cells, they also primed CD8+ CTL specific for the co-administered antigen [44] (Simmons et al. 1999), irrespective of the route used for immunization. If CT and CT mutants appear to activate predominantly Th2-type responses, with a preferential induction of Th1-type functional phenotype, as evidenced by the preferential induction of antigen-specific IgG1 antibody and of Th2-type cytokines such as IL-4 and IL-5, using a variety of model vaccines [57].

In mice, MF59 fully restored the ability of old mice to mount a strong antibody response to flu vaccine equal to that observed in young, fully immunologically competent mice.

2.2.3 Emulsion Adjuvants

We are worked extensively with Montanide ISA 720 and only occasionally with Montanide ISA 51. These two adjuvants are available upon request to Air Liquid/Seppic, Paris, France. These water-in-oil emulsions contain a degradable mineral oil (ISA 51) or a vegetable oil (ISA 720). Our choice on ISA 720 was dictated by the fact that it was easier to inject in animals, but in terms of an immune response, the two are comparable at least with the few antigens we have used. The antibody response tends to increase rapidly and second injections may not be needed at least at the antigen concentrations we have used (10-50 µg/mouse). Besides antibody responses to numerous long synthetic malaria antigens, we could obtain CD8+ T cells specific for various antigens including short peptides corresponding to specific CTL epitopes [50].

Due to the excellent response obtained with P. falciparum CSP C-terminal fragment 282-383 in pre-clinical studies, a clinical study was designed to assess the immunogenicity of this fragment [51]. The LSP was formulated in alum and Montanide ISA 720 at the two doses of 100 and 300 micrograms and injected intramuscularly. The preparation was well tolerated, and only few minor adverse reactions were recorded. High sporozoite-specific antibody titers were elicited in the volunteers who received the Montanide formulation at the 100µg antigen dose. In addition, robust lymphocyte proliferation responses were also elicited with concomitant production of IFN-γ, crucial in the elimination of the parasite in almost all volunteers. Most importantly, as seen in mice, we also observed the development of CD8+ T lymphocyte responses, which are important in the immunity to malaria. This was the first example in which a malaria candidate could simultaneously activate specific CD4+, CD8+ T and B cells.

A new phase I clinical trial using a GMP preparation was then designed to determine the optimal dose and adjuvant, which will be tested for efficacy studies using live sporozoites in a phase II clinical trial. Results from both clinical studies should be available at the end of 2004.

Both Montanide ISA 51 and 720 have been tested in animals and thousands of individuals and found to be safe [52-56]. Nevertheless, the proper antigen concentration has to be established. Adverse effects are usually depending on the concentration and nature of the antigen.

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Developed by Chiron, MF59 is an oil-in-water emulsion consisting of uniformly small and stable droplets of the natural and fully metabolizable oil squalene, stabilized by addition of emulsifiers. The adjuvant is very stable and highly versatile since it can be formulated with antigens with diverse characteristics. The fine mechanisms of the adjuvanticity of MF59 are not understood yet. MF59 preferentially polarizes the immune response towards a Th2-type functional phenotype, as evidenced by the preferential induction of antigen-specific IgG1 antibody and of Th2-type cytokines such as IL-4 and IL-5, using a variety of model vaccines [57].

In mice, MF59 fully restored the ability of old mice to mount a strong antibody response to flu vaccine equal to that observed in young, fully immunologically competent mice.
consequently, prolonged immune responses after a single dose of vaccine were obtained using microencapsulated antigens. PLGA microspheres were shown to induce up to 127-fold increase of the immunogenicity of a HBV vaccine containing the preS2 region as compared to alum-adjuvanted vaccine, with protective antibody titers persisting for several months [60]. Similarly, in baby baboons it strongly enhanced the protective (bactericidal) antibody response after immunization of conjugate vaccines against Haemophilus influenzae type b (Hib) and against Neisseria meningitidis group C (MenC) [61]. Finally, MF59 induced a strong and persisting antibody response against recombinant FimH from uropathogenic E. coli in cynomolgus monkeys, which were protected against bacteriuria and pyuria induced by an infectious challenge [62].

When given to elder people, the MF59 trivalent subunit vaccine against influenza exhibited a very good safety profile. A consistent finding of the several clinical trials carried out was an increased immunogenicity of the MF59-adjuvanted vaccine as compared to the conventional vaccine, which was observed even after repeated immunizations. A significant increased immunogenicity of the MF59-adjuvanted vaccine was observed in elder subjects with chronic diseases, such as respiratory and cardiovascular diseases, and diabetes mellitus. The use of MF59 increased the immunogenicity to heterovariant strains of the influenza virus [63]. More recently, MF59 was shown to dramatically enhance the immunogenicity to an A/H5N1 antigen, from a potentially pandemic strain of influenza virus [64]. In addition, the induced antibody persisted for a much longer time and were significantly boosted by a secondary immunization 16 months after the first [65]. The MF59-adjuvanted subunit vaccine against influenza is now licensed and available in most European countries.

The clinical experience with the MF59 adjuvant is much wider than its use with the influenza vaccine. MF59 has been tested in formulation of vaccines against HBV, hepatitis C virus (HCV), herpes simplex virus (HSV), human papillomavirus (HPV), cytomegalovirus (CMV), human immunodeficiency virus-1 (HIV-1), and is being also tested in humans with bacterial vaccines [57]. MF59 was shown to strongly enhance the immunogenicity of an HBV vaccine containing the preS2 region, conferring seroprotection to 89% of vaccinated subjects already after the first dose of the vaccine [66]. Importantly, recombinant proteins from CMV and HIV-1 formulated with MF59 and given to toddlers (second year of life) and to newborns (within the first three days of life) were more immunogenic and safer than the same vaccines given to adults [67-70]. Furthermore, the use of the MF59 adjuvant allowed to reduce significantly the amount of vaccine required to induce high antibody and cellular immune responses, as compared to the same vaccine given with alum as adjuvant [67].

2.2.4 Microencapsulated Antigens

The microencapsulation of antigens into biodegradable poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) to obtain sustained antigen delivery and, consequently, prolonged immune responses after a single injection was proposed by the WHO in the late 1980ies [71,72]. Antigen release from PLA and PLGA microspheres is partly governed by the biodegradation of the polymers, which in turn, is a function of the copolymer composition and molecular weight. Thus, antigen release pattern and kinetics are controllable by the selection of appropriate polymer types [71,73,74]. A wealth of publications demonstrates the large interest for this delivery/adjuvant system, through which sustained and high antibody together with helper and cytotoxic T cell responses can be obtained [33, 75-77]. Microcapsules are readily taken up by professional antigen presenting cells with concomitant activation of murine and human CTL clones up to 9 days [78]. These properties should be best exploited in tumor vaccine development based on CTL. In spite of these promising results, only very few clinical trials have been initiated with biodegradable microspheres. In our opinion, this situation essentially derives from a technical processing difficulty and a misperception. In fact, a major stumbling block in the manufacturing of microcapsules for vaccine trials is the aseptic environment, in which the antigen encapsulation has to take place. This requires dedicated facilities with little adaptability to production of relative small GMP quantities needed for phase I clinical trials. Thus, only major pharma companies can possibly afford this process. Efforts to obviate this problem have been undertaken and it is now feasible to produce small scale GMP vaccine in biodegradable microcapsule formulations at limited cost (Dr B. Gander, personal communication). The other factor resides in the fact that early publications indicated that antigens may be destroyed by the acidic environment created by the hydrolysis of PLA and PGLA. While this was observed with tetanus toxoid microparticles [79], we could not determine any degradation when long synthetic peptides were encapsulated. It is, therefore, tempting to speculate that the degradation of tetanus toxoid was due to the presence of impurities (proteolytic enzymes) in the toxoid preparation (only 50% is tetanus toxoid) or this phenomenon is restricted to only a few cases. In addition, PLA/PGLA microcapsules containing human growth hormone are commercially available, again indicating that the product is stable or can be stabilized [80]. Recently, it has been shown that it is possible to microencapsulate together several antigens (tetanus toxoid, diptheria toxoid, Hib and P. falciparum CSP) in a single formulation. Modulation of the specific responses are observed. Variation of antibody responses from single antigen encapsulation is composition and animal dependent [81]. It is our aim to conduct clinical trials in the future by using the microencapsulation technology.

3. CONCLUSIONS

As discussed above, researchers working on vaccine development have now the choice of a plethora of adjuvants with different properties. It is, therefore, of fundamental importance for vaccine development to exactly know the type of protective response one aims to obtain. The discovery of TLR will certainly generate in the future new powerful molecules act to activate or down-regulate specific TLR such that a precise tailor-made protective immune response is obtained. Thus, through recent and future adjuvant
discoveries, it is not too optimistic to think that infectious diseases of world-wide concern will be finally eradicated in a not so far future.

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