Review

ISCOMATRIX: a novel adjuvant for use in prophylactic and therapeutic vaccines against infectious diseases

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The ISCOMATRIX adjuvant has antigen delivery and presentation properties as well as immunomodulatory capabilities, which combine to provide enhanced and accelerated immune responses. The responses are broad, including a range of subclasses of antibodies as well as CD4+ and CD8+ T-cells. A range of ISCOMATRIX vaccines (ISCOMATRIX adjuvant combined with antigen) have now been tested in clinical trials and have been shown to be generally safe and well tolerated as well as immunogenic, generating both antibody (Ab) and T-cell responses. The mechanisms by which ISCOMATRIX adjuvant facilitates its immune effects are the scope of significant study and indicate that ISCOMATRIX adjuvant (i) rapidly traffics antigen into the cytosol of multiple dendritic cell subsets, (ii) induces the induction of an array of cytokines and chemokines and (iii) links the innate and adaptive immune responses in vivo in a Toll-like-receptor-independent but MyD88-dependent manner. These data highlight the clinical utility of ISCOMATRIX adjuvant in the development of prophylactic and therapeutic vaccines for infectious disease.

Introduction

Prophylactic vaccination against pathogens has contributed greatly to improving human health. No better examples can be demonstrated than the effective control of polio, smallpox, measles, diphtheria, tetanus and rabies. However, there is still an urgent need for vaccines against several pathogens that cause serious human infections and increased morbidity. Some of these include human immunodeficiency virus (HIV), Mycobacterium tuberculosis, Plasmodium falciparum, hepatitis C virus (HCV), respiratory syncytial virus and dengue virus (Aurisicchio & Ciliberto, 2010; Leroux-Roels, 2010). The most successful vaccines against pathogens have tended to be live, attenuated or inactivated whole-cell vaccines, which often have inherent pathogen-derived adjuvants or pathogen-associated molecular patterns (PAMPs) which are strongly responded to by the immune response through pattern recognition receptors (PRRs). Although efficacious, such vaccines are also associated with increased reactogenicity. The newer generation of vaccines are, typically, derived from purified pathogen subunits to reduce overt reactogenicity, but as a result are also less efficacious and require the addition of exogenous adjuvants to reinstate immunogenicity.

Adjuvants provide additional signals during the induction of immune responses to the vaccine antigen with the aim of inducing either more rapid and robust immune responses that correlate with increased protection or allowing for dose-sparing in the context of antigens, which can be in limited supply or problematic to manufacture. In addition, adjuvants are thought to be critical for vaccination of chronically infected individuals, as they can overcome the pathogen’s immune evasion mechanisms such as immunosuppression and/or reprogramming of the immune response. Adjuvanted vaccines also provide a rational strategy to induce protection in individuals whose immune system is waning or dysfunctional due to senescence or immune suppression. Immune protection during a pandemic outbreak requires the rapid induction of immunity. In such situations, adjuvanted vaccines that are able to induce cross-reactivity among same-species pathogens provide a significant advantage. Finally, adjuvants lower the threshold of activation of the immune system, so that it will respond to a low dose of antigens, and/or fewer immunizations, which reduces the cost of vaccine manufacturing.

Abbreviations: Ab, antibody; APC, antigen presenting cells; AS, adjuvant systems; DC, dendritic cells; DLN, draining lymph nodes; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HPV, human papillomavirus; IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; MPL, monophosphoryl lipid A; NK, natural killer; ODN, oligodeoxynucleotide; PAMP, pathogen-associated molecular pattern; poly(I: C), polyinosinic:polycytidylic acid; PRR, pattern recognition receptor; Th, T-helper; TIV, trivalent influenza vaccine; TLR, Toll-like receptor.
and facilitates broader vaccination. Adjuvant development is an active area of research driven by the need to improve current vaccines and develop new vaccines against infectious diseases.

**Mechanism of defence against infection**

The initiation of the immune response against pathogens involves recognition of PAMPs and activation of innate cells and dendritic cells (DCs) through PRR signalling. These include cell-surface and endosomal Toll-like receptors (TLRs), surface C-type lectin receptors and the cytosolic NOD-like receptors and RIG-I-like receptors (Takeuchi & Akira, 2010). The cytokines secreted by DCs direct the differentiation of CD4+ T-helper (Th) cells into subpopulations (i.e. Th1, Th2, Th17) differing in their cytokine profile and effector functions. The production of interleukin (IL)-12 by DCs promotes differentiation of Th1 cells [secreteting interferon (IFN)-γ, IL-2 and tumour necrosis factor (TNF)-α], which activate natural killer (NK) cell and macrophage functions and induce IFN-γ-regulated Ab isotypes that enhance phagocytosis, Ab-dependent cell cytotoxicity and complement activation. In contrast, IL-4 promotes Th2 cell differentiation (IL-4, IL-5 and IL-6), which activates mast cell and eosinophil functions as well as IgE and IgG1 Ab responses. In general, Th1 responses play critical roles in the protection against intracellular pathogens, whereas Th2 responses are involved in the eradication of extracellular pathogens (Kawai & Akira, 2010). Th17 cells secrete cytokines such as IL-17 and IL-22 and are involved in pro-inflammatory responses against extracellular pathogens. They are also associated with inflammatory immunopathologies in some individuals (e.g. allergy, inflammatory bowel diseases) or autoimmune (Korn et al., 2009).

**Evasion of the immune response by pathogens**

Pathogens have evolved mechanisms to evade the immune response. Viruses, such as HCV or influenza virus, degrade TLR signalling molecules (Li et al., 2005) or inactivate RNA receptors (Pichlmair et al., 2006). Similarly, species of the genera *Brucella* and *Ochrobactrum* minimize pathogen detection by PRRs by expressing modified LPS (Lapaque et al., 2005). Epstein–Barr virus, human cytomegalovirus and some bacteria interfere with antigen processing and presentation, reducing the magnitude of the adaptive response. Furthermore, certain HCV proteins induce T regulatory cells that suppress emerging effector T-cell responses (Li et al., 2009). *Porphyromonas gingivalis* and *Listeria monocytogenes* avoid detection by being retained within phagosomal compartments (Birmingham et al., 2008; Dorn et al., 2001) and *Brucella anthracis* avoids detection by inducing cell apoptosis (Park et al., 2002). Therefore, the selection of adjuvants for any given disease must take into consideration the immune evasion mechanisms of the pathogen, the immunological mechanisms required to achieve protection and whether the type of immune response induced by the adjuvant fulfils these parameters.

**Approved and novel adjuvants**

Very few adjuvants are currently licensed for human vaccine use. The most common are the aluminium salts which have been used as vaccine adjuvants for over 80 years in a very large number of individuals, demonstrating a good safety profile. Although aluminium salts are effective for induction of Ab responses, generation of Th2 responses and, more recently, activation of the inflammasome (Kool et al., 2008), they are not noted for induction of CD8+ T-cell responses and often require high amounts of antigen to generate effective immunity (Kuroda et al., 2011). The adjuvant MF59, developed by Novartis, is an oil-in-water emulsion that is believed to stimulate chemokine production by monocytes, macrophages and granulocytes and activates an array of cytokines and host defence pathways at the injection site (Seubert et al., 2008; Mosca et al., 2008). MF59 has been licensed for an influenza vaccine for the elderly (Fluad, Novartis) and also enhances the efficacy of a seasonal influenza vaccine for young children (Podda, 2001). Clinical studies showed that the H5N1–MF59-adjuvanted vaccine induces cross-reactive immune responses among different viral clades, which might be important during pandemics (Banzhoff et al., 2009). MF59 has been administered to large numbers of individuals and has a good safety profile, and although some Th1 responses are induced, there are no detectable CD8+ T-cell responses reported.

The need for more potent Ab responses, as well as generation of Th1 responses, in particular CD8+ cytotoxic T-cell responses, has resulted in continued investigations into identifying novel adjuvants with these specific features (O’Hagan et al., 2011). The discovery of PRRs and their role in the initiation of immune responses has led to the development of PRR agonists as either individual adjuvants or as components of adjuvant combinations. Monophosphoryl lipid A (MPL) and QS-21 (saponin from *Quillaja saponaria* tree), developed by GlaxoSmithKline, are the basis for the adjuvant systems (AS) AS01 and AS04. AS01 contains MPL and QS-21 as a liposome, AS03 contains α-tocopherol and squalene in an oil-in-water emulsion and AS04 contains MPL and aluminium salt (Garçon & Van Mechelen, 2011). Clinical trials are in progress with AS01 vaccines for HIV and tuberculosis. A Phase III clinical trial with AS01 vaccine showed protection against clinical and severe malaria in children (Agnandji et al., 2011). AS03 has been included in a pandemic influenza vaccine (Leroux-Roels et al., 2007) and a hepatitis B virus vaccine, and AS04 has been used in a human papillomavirus (HPV) vaccine. In both cases, Ab levels were enhanced compared to those with antigen formulated with aluminium salts alone (Beran et al., 2005; Paavonen et al., 2009). Bacterial unmethylated CpG oligodeoxynucleotide (ODN) is a TLR-9 agonist that induces Th1 cytokines (Bode et al., 2011) and in murine
models is able to protect against anthrax, leishmania, influenza virus, measles virus, lymphocytic choriomeningitis virus, orthopox virus and hepatitis B virus infections (Klinman et al., 2009). CpG ODN improves Ab responses when co-administered with Engerix B, pneumococcal conjugate vaccine or 23-valent pneumococcal polysaccharide vaccine to HIV-infected subjects and is currently in Phase III clinical studies (Bode et al., 2011; Cooper et al., 2004). CpG is also included as a component of the AS15 adjuvant complex that also contains MPL and QS21 (Brichard, 2005). To date, although CD4+ T-cell responses have been readily detected with the AS series of adjuvants, CD8+ T-cell responses are less frequently reported (Sun et al., 2003).

The TLR-3 ligand polyinosinic:polycytidylic acid [poly(I: C)] induces T-cell responses in animal models, although data from non-human primates showed limited success due to degradation of poly (I: C) by serum proteases (Li et al., 2011; Steinhagen et al., 2011). Derivatives of poly(I: C), such as poly ICLC (Ampligen), are under evaluation in clinical trials (Jasani et al., 2009). Liposome encapsulation of poly ICLC reduces its toxicity and enhances the duration of protection of an influenza virus vaccine in mice, highlighting the link between vaccine formulation and its efficacy and safety profile (Li et al., 2011; Tewari et al., 2010; Wong et al., 2005). The TLR-5 agonist flagellin enhances the immunogenicity of poorly immunogenic antigens when chemically linked. Protective Ab responses were observed in mice vaccinated against Yersinia pestis, West Nile virus, vaccinia virus, influenza virus, Pseudomonas aeruginosa and parasites (Campodónico et al., 2011; Mizel & Bates, 2010). Flagellin conjugated with haemagglutinin from H1N1 influenza virus has been shown to be safe and to induce sero-protection in the elderly (Taylor et al., 2011).

Imidazoquinolines such as imiquimod (R848) or resiquimod (R837) are double cyclic organic molecules that act as TLR-7/8 agonists. Imiquimod (Aldara, 3M) has been licensed for topical use in HPV infections and certain skin cancers (e.g. basal cell carcinoma). In combination with FLT3 ligand, imiquimod enhances the CD8+ T-cell response against melanoma antigens in humans and simian immunodeficiency virus antigens in non-human primates (Kwissa et al., 2007). TLR-8 agonists can also enhance immunogenicity in mice to recombinant hepatitis B antigen in comparison to aluminium-containing adjuvants (Du et al., 2010).

**ISCOMATRIX adjuvant**

The name ISCOMATRIX derives from the ‘immunostimulating complex’ or ‘ISCOM’, which was first described by Morein and colleagues in 1984 (Morein et al., 1984) and was formed by combining saponin, cholesterol, phospholipid and hydrophobic antigens. The classical ISCOM technology, however, required incorporation of vaccine antigens into the structure, which not only restricted the types of antigens that could be used but was a complex process that was difficult to control. We have addressed these issues with the development of the ISCOMATRIX adjuvant which does not require antigen to be incorporated and is manufactured by a relatively simple, well controlled process (Pearse & Drane, 2007). The ISCOMATRIX adjuvant contains purified fractions of Quillaia saponaria extract (ISCOPREP saponin), cholesterol and phospholipid, which combine under controlled conditions to form cage-like structures, typically 40–50 nm in diameter (Fig. 1). All the components used in the manufacture of ISCOMATRIX adjuvant are synthetic or plant derived and the adjuvant can be simply mixed with virtually any antigen to formulate the required vaccine. Furthermore, the components have been extensively refined and developed since 2000 to produce an optimized formulation capable of meeting the stringent needs for human vaccines in the 21st century.

**Mechanism of action of ISCOMATRIX adjuvant**

Understanding the mode of action of novel adjuvants is now essential in defining adjuvant safety and potential applications to support registration of the final vaccine product. We have used a wide range of in vivo and in vitro approaches as well as gene profile analyses of highly purified cell populations from the draining lymph nodes (DLN) and blood of vaccinated animal models to identify which cell populations are activated, which signalling pathways are activated, how these are involved in the innate and adaptive immune responses and how DCs coordinate these interactions in vivo. A summary of our findings is represented in Fig. 2 showing that ISCOMATRIX adjuvant is effective at both antigen delivery and immune-stimulation and, as such, should be considered an integrated adjuvant system.

**ISCOMATRIX adjuvant in prophylactic vaccines**

![ISCOMATRIX adjuvant](image-url)
Antigen delivery occurs when antigen–ISCOMATRIX vaccine particles traffic directly into DLN within the first 2 h following injection where they are taken up by resident DCs and other APCs. DCs from the injection site also traffic captured ISCOMATRIX vaccine to the DLN by 24–48 h post-inoculation, thus providing two waves of antigen presentation. DCs internalize ISCOMATRIX vaccines via actin-dependent uptake into endosomes or phagolysosomes. During acidification of these compartments, ISCOMATRIX adjuvant facilitates antigen translocation into the cytosol, a prerequisite for access to the class I MHC pathway for cross-presentation to CD8+ T-cells. Antigen is also made available for loading onto class II MHC for presentation to CD4+ T-cells and induction of B-cell responses. Antigen presentation within the DLN is sustained for up to 7 days (intracellular depot within LN-resident DCs as well as newly arriving injection site DCs), ensuring continuous and prolonged antigen presentation. Immune-stimulation occurs via the induction of cytokine and chemokine cascades within 6 h, which facilitate activation of DCs and other innate immune effectors and trigger their recruitment into the DLN. Lymph nodes shut down leukocyte efflux for 24 h, retaining immigrating cells and increasing cellular interactions of APCs with B-cells, T-cells and other innate effectors ensuring antigen presentation and development of effector function. The co-expression of these two functional facets ensures the generation of robust adaptive immune responses. In this way, ISCOMATRIX adjuvant can be considered an integrated adjuvant system.

Although some of the features depicted in Fig. 2 overlap with those observed with other adjuvants (e.g. induction of cytokines/chemokines, CD4+ T-cell responses and Ab responses) there are also major differences noted (also shown in Fig. 3). One difference is that ISCOMATRIX adjuvant induces both Th1 and Th2 responses (and IFN-γ and IL-5 are rapidly but transiently detected in serum) (Wilson et al., 2011). This results in both robust and effective Ab and CD8+ T-cell responses. Generation of high-frequency antigen-specific CD8+ T-cell responses is a reproducible feature of ISCOMATRIX vaccines in animals and humans (Davis et al., 2004; Ebert et al., 2009; Drane et al., 2009; Nicholaou et al., 2011), as this is not readily (or weakly at best) detected with other adjuvant systems.

The first sign of immune cell reaction observed in mice inoculated with ISCOMATRIX or ISCOM vaccines is an increase in the number of NK cells, B-cells, T-cells, DCs and granulocytes detected within the lymph nodes at 6 h and peaking by 24–48 h following vaccination. This is a transient and reversible efflux of cells, declining to normal levels by 72 h post-vaccination (Wilson et al., 2011; Duewell et al., 2011). The activation of NK cells appears to be under the regulation of DCs and specific cytokines, such as TNF-α and IL-18. Activation of innate cells may explain the rapid (peak by 6 h) but transient (basal levels by 24 h) induction of an array of serum cytokines and chemokines following vaccination (Wilson et al., 2011). These include pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6) as well as chemokines and cytokines involved in macrophage and NK cell activation (e.g. CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES), CXCL9 (IP-10), CXCL10 (IP-10), CXCL11 (I-TAC), and CXCL13 (BCA-1)).
DCs and peripheral blood-derived DCs (Schnurr et al., 2011; unpublished data). It is of note that NK cell activation is not seen with other adjuvant systems such as aluminium salts (Fig. 3a) and has not been reported for other adjuvant systems.

Unlike other adjuvant systems, ISCOMATRIX vaccines rapidly traffic to the DLN within the first 2 h after injection (as assessed by fluorescently tagged ISCOMATRIX adjuvant), loading lymph node-resident DCs and other antigen presenting cells (APCs). In parallel, antigen presentation is detected in the DLN as early as 12 h post vaccination (Wilson et al., 2011). This is in contrast to other adjuvant systems that predominantly remain within the injection site and require uptake locally by innate immune effectors or APCs such as CD11b+ monocytes for transportation to the DLN (Mosca et al., 2008). Eventually, injection site DCs and APCs will also traffic captured ISCOMATRIX vaccine from the injection site into the DLN, but their contribution occurs later (24–48 h). Together, these two waves of vaccine antigen trafficking (direct trafficking of adjuvant and via peripheral APC-transport) result in prolonged antigen presentation.

Given the importance of DCs in the activation of adaptive immune responses, we have carried out detailed analyses to determine which DC subpopulations are involved in cross-presenting antigens to CD8+ T-cells in vivo using OVA–ISCOMATRIX or pOVA–ISCOM vaccines. Duevell et al. (2011) showed that conventional DCs, but not Langherin+ DCs, cross-present antigen i.e. they present exogenously derived antigens onto class I major histocompatibility complex (MHC) to CD8+ T-cells instead of class II MHC to CD4+ T-cells and prime CD8+ T-cells. In addition, cross-presentation of antigen in vivo persists for up to 7 days after priming, which, together with efficient antigen delivery, provides a mechanistic explanation for the strong CD8+ T-cell responses induced by p-OVA–ISCOM vaccine. The same study shows that the ISCOM vaccine is more efficient at inducing cytotoxic T-lymphocyte responses in vivo than other adjuvants such as aluminium hydroxide, incomplete Freund adjuvant, CpG, LPS or Pam3Cys (Duevell et al., 2011). This is also shown in Fig. 3(b), where ISCOMATRIX adjuvant is shown to be superior at inducing OVA-specific IFN-γ+CD8+ T-cell responses as compared to aluminium hydroxide or the TLR3 agonist poly(I:C). In a similar study using an OVA–ISCOMATRIX vaccine, Wilson and colleagues showed that CD8x+ and migratory-type DCs cross-present antigen in vivo (Wilson et al., 2011). In vitro experiments with human DCs have also shown efficient cross-presentation of the cancer–testes tumour antigen NY-ESO-1 when formulated with ISCOMATRIX adjuvant by both monocyte-derived DCs and peripheral blood-derived DCs (Schnurr et al., 2009).

Perhaps the most intriguing aspect of the mechanism of action of ISCOMATRIX adjuvant is which components are recognized by the immune system and how this translates into adaptive immune responses. To date, our studies indicate that TLRs, per se, are not involved in recognition of the ISCOMATRIX adjuvant cage-like structure or its components. The activation of the inflammasome has been suggested as a mechanism of action of particulate adjuvants (Sharp et al., 2009). Although we do not have conclusive evidence to support inflammasome activation by ISCOMATRIX adjuvant in vivo, there is strong evidence that Nalp3 and other inflammasome components are involved in vitro (unpublished data). Furthermore, IL-1β is induced in vivo (Wilson et al., 2011), suggesting a link to the inflammasome in vivo. We are currently investigating the molecular pathways that lead to secretion of IL-1β/IL-18 and the potential role of the various inflammasome components in this process. Evidence, to date, suggests a major role for IL-18 in the ISCOMATRIX adjuvant-induced adaptive immune responses (unpublished data).

Induction of T-cell responses by ISCOMATRIX vaccines

ISCOMATRIX adjuvant consistently stimulates robust and readily detectable CD4+ and CD8+ T-cell responses to various antigens in mice and humans. Mice vaccinated twice with OVA-ISCOMATRIX vaccine show expansion and differentiation of specific CD8+ T-cells expressing IFN-γ and TNF-α (Duevell et al., 2011). Effector responses are followed by differentiation of functional memory CD8+ T-cells which persist up to 1 year after priming (unpublished data). Induction of T-cell responses was demonstrated in a mouse model of B16 tumour cells expressing NY-ESO-1. Association of NY-ESO-1 with ISCOMATRIX adjuvant induces strong IFN-γ responses and cytolytic CD8+ T-cells (Maraskovsky et al., 2004). Stimulation of T-cells by antigens delivered with ISCOMATRIX adjuvant has also been shown in human cells and non-human primates. Robson et al. (2003) showed that ISCOMATRIX adjuvant delivers OVA antigen in vitro to dendritic cells and primes CD4+ and CD8+ T-cells (Robson et al., 2003). Broad responses including, Ab, CD4+ and CD8+ T-cell responses were generated in rhesus macaques vaccinated with HCV core antigen and ISCOMATRIX adjuvant (Polakos et al., 2001). Vaccination of patients with recombiant HPV 16 early gene proteins E6 and E7 (HPV-16E6/E7) formulated with ISCOMATRIX adjuvant consistently elicited both high titre Ab and specific cytotoxic T-cells in healthy and HIV-infected individuals (Anderson et al., 2009; Frazer et al., 2004). Similarly, T-cell responses were detected in the majority of individuals receiving an HCVcore/ISCOMATRIX vaccine (Drane et al., 2009). Furthermore, similar broad epitope immune responses were generated in cancer patients expressing NY-ESO-1+ tumours and these were sustained for several years (Davis et al., 2004; Ebert et al., 2009; Nicholaou et al., 2011).
Induction of Ab responses by ISCOMATRIX vaccines

Induction of CD4+ T-helper cell responses is a prerequisite for induction of vaccine-specific Ab responses. Prophylactic vaccines need to induce high-titre Ab responses that are long-lived, of high affinity and of isotypes capable of activating appropriate immune effector functions. Several studies have now shown that ISCOMATRIX vaccines induce high and persistent Ab responses, which suggests the induction of long-lasting plasmocytomas and B-cell memory as described by Vaughan et al. (2011). We have shown that, in mice, an OVA-ISCOMATRIX vaccine induces formation of persistent germinal centres and plasmocytomas, both of which explain the long-lasting Ab responses observed in mice vaccinated with ISCOMATRIX vaccines. Furthermore, the induction of high and persistent Ab titres is detectable in blood up to 1 year after one or two vaccinations, as well as significant affinity maturation (unpublished data). Similar findings were reported by Clements et al. (2010), in mice vaccinated with DEN2-80E recombinant antigen from dengue virus together with ISCOMATRIX adjuvant. In the same study, several adjuvants were compared and ISCOMATRIX adjuvant was the only one that induced high titres of neutralizing antibodies at a very low dose of antigen, either in mice or non-human primates (Clements et al., 2010). Enhanced and persistent Ab repertoire was also generated in cancer patients receiving the NY-ESO-1/ISCOMATRIX vaccine but not NY-ESO-1 protein alone, confirming these Ab-inducing features of ISCOMATRIX adjuvant (Mischo et al., 2011). The isotype of the vaccine-induced Ab responses determines their effector functions; this isotype switching process is regulated by the cytokines secreted by Th-cell subpopulations (Th1, Th2, Th17). ISCOMATRIX adjuvant induces a mixed Th1/Th2 cytokine response, promoting the expression of several Ab isotypes (Sjölander et al., 1997). It is of note that subsequent analyses of the humoral immune response using RAYS technology from cancer patients receiving the NY-ESO-1 ISCOMATRIX vaccine (but not NY-ESO-1 protein alone vaccine) indicated that the NY-ESO-1 ISCOMATRIX vaccine induced increased Ab titres and cross-reactivity to the related cancer–testes antigen LAGE 1A and B-related antigens with a preference for human IgG1 and IgG3 (murine IgG2a and IgG2b) rather than IgG4 (mouse IgG1) subclasses, preferentially targeting the N-terminal sequence of NY-ESO-1 (Mischo et al., 2011). These isotypes are cytophilic and complement fixing and are beneficial for eradicating target cells. This capability distinguishes ISCOMATRIX adjuvant from other adjuvant systems, such as alum, which preferentially generate the human IgG4 isotype (murine IgG1).

Dose-sparing benefits of ISCOMATRIX vaccines

ISCOMATRIX adjuvant has also been able to generate robust Ab responses at lower antigen doses in several antigen model systems, suggesting a capacity for dose sparing (Pearse & Drane, 2004). A significant dose-sparing effect was reported in a mouse model for influenza, where similar Ab titres to those induced with split MEM71 influenza virus were achieved with ISCOMATRIX vaccine containing 100-fold less antigen (Sanders et al., 2009). Enhanced immunogenicity at a low dose of antigens was also observed using recombinant gp120 in guinea pigs (Boyle et al., 2007).

Route of administration options for ISCOMATRIX vaccines

The route of inoculation is an important consideration in vaccine development and immunization protocols as it can determine the type and quality of the immune response. In this regard, ISCOMATRIX vaccines are versatile, with several studies showing they can be administered via both parenteral and non-parenteral routes. Coulter et al. (2003) showed that the Ab responses induced by an ISCOMATRIX influenza vaccine delivered intranasally, were higher than the responses induced by the unadjuvanted vaccine when delivered subcutaneously (Coulter et al., 2003). A clinical evaluation with an influenza ISCOMATRIX vaccine delivered intranasally showed that it was possible to induce mucosal and systemic Ab responses simultaneously (Drane et al., 2007). Such versatility was also observed in a mouse model for Helicobacter pylori infection where both ISCOM and ISCOMATRIX vaccines induced protection in two genetic backgrounds of mice, using two different antigens and two different delivery routes (Skene et al., 2008). Finally, a recent study has shown that pulmonary delivery of an influenza ISCOMATRIX vaccine also induced strong systemic and mucosal Ab responses (Vujanic et al., 2010). Thus, there are several route options for delivery of ISCOMATRIX vaccines that need to be explored clinically to determine whether the traditional intramuscular route is sufficient for all ISCOMATRIX vaccines or whether there are merits/advantages to targeting alternate routes in specific cases.

ISCOMATRIX adjuvant can be combined with other adjuvants

The finding that ISCOMATRIX adjuvant does not act via specific TLRs but does involve the MyD88 pathway suggests that there are benefits of combining ISCOMATRIX adjuvant with TLR agonists or other adjuvants, thus simultaneously engaging multiple signalling pathways. In this regard, Jacobs et al. (2011) recently reported that combining ISCOMATRIX adjuvant with CpG ODN not only generated an increased magnitude CD8+ T-cell response, it also overcame tumour-induced immune suppression in a pancreatic cancer model, resulting in regression of established cancer where either adjuvant alone failed. We have found synergies when combining ISCOMATRIX adjuvant with other TLR agonists as well as with aluminium salts for both Ab responses and CD4+ or CD8+ T-cell responses (unpublished data).
Clinical studies involving ISCOMATRIX vaccines

CSL has completed six clinical studies using optimized formulations of the ISCOMATRIX adjuvant. These studies were completed as part of three separate development programs for a therapeutic HPV vaccine, a therapeutic HCV vaccine and a prophylactic influenza vaccine. A comprehensive analysis of the clinical safety data for ISCOMATRIX adjuvant has recently been reported (McKenzie et al., 2010). Briefly, clinical evaluation of the local and systemic reactogenicity profile of ISCOMATRIX adjuvant has shown that it is safe and generally well-tolerated in all populations studied and in all antigen–adjuvant combinations. Local reactions were predominantly mild to moderate and resolved within 4 days, and a flu-like syndrome was the most common unsolicited systemic adverse effect. No evidence of allergic or autoimmune disorders was observed. Between CSL and our partners, there have been 16 completed or ongoing clinical studies with ISCOMATRIX vaccines. Approximately 1600 subjects have received at least one dose of either an ISCOMATRIX vaccine or the adjuvant alone.

A study to determine safety, tolerability and immunogenicity of an HCV core protein ISCOMATRIX vaccine demonstrated the vaccine was safe and well-tolerated, and generated robust Ab responses to the HCV core protein with T-cell cytokine responses detected in the majority of vaccines (Drane et al., 2009). A study to determine safety, tolerability and immunogenicity of a split virion trivalent influenza vaccine (TIV) formulated with ISCOMATRIX adjuvant in healthy adults and the elderly (>65 years) has been performed to assess whether the ISCOMATRIX-formulated TIV could enhance vaccine immune responses in the elderly where immune senescence is a major issue. A range of different doses of antigen and ISCOMATRIX adjuvant were tested in ~400 volunteers. The ISCOMATRIX vaccine generated higher increases in geometric mean titres than the unadjuvanted TIV in the elderly in response to all three influenza strains. In addition, strong CD4+ T-cell responses were induced which approximated responses seen with the younger adults, suggesting that ISCOMATRIX adjuvant has the potential to overcome immune senescence in the elderly (unpublished data).

Conclusion

In summary, ISCOMATRIX adjuvant possesses dual functions: efficient antigen delivery and immune stimulating properties, which combine to induce potent and persistent effector and memory Ab and cellular responses. These properties, together with an acceptable safety profile and a well-controlled large-scale manufacturing process, support the use of ISCOMATRIX adjuvant for development of therapeutic and prophylactic human vaccines against intra- or extracellular pathogens. There is also scope for combining ISCOMATRIX adjuvant with other adjuvant systems (e.g. TLR agonists) that should further enhance the magnitude of the immune responses generated. Clinical studies will provide information as to the best settings for using ISCOMATRIX adjuvant in humans.

References


