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Review

ISCOMs and ISCOMATRIX™

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ABSTRACT

Immunostimulating complexes (ISCOMs) are particulate antigen delivery systems composed of antigen, cholesterol, phospholipid and saponin, while ISCOMATRIX™ is a particulate adjuvant comprising cholesterol, phospholipid and saponin but without antigen. The combination of an antigen with ISCOMATRIX™ is called an ISCOMATRIX™ vaccine. ISCOMs and ISCOMATRIX™ combine the advantages of a particulate carrier system with the presence of an invisible adjuvant (Quil A) and consequently have been found to be more immunogenic, while removing its haemolytic activity of the saponin, producing less toxicity. ISCOMs and ISCOMATRIX™ vaccines have now been shown to induce strong antigen specific cellular or humoral immune responses to a broad range of antigens of viral, bacterial, parasite origin or tumor in a number of animal species including non-human primates and humans. These vaccines produced by well controlled and reproducible processes have also been evaluated in human clinical trials. In this review, we summarize the recent progress of ISCOMs and ISCOMATRIX™, including preparation technology as well as their application in humans and veterinary vaccine designs with particular emphasis on the current understanding of the properties and features of ISCOMs and ISCOMATRIX™ vaccines to induce immune responses. The mechanisms of adjuvanticity are also discussed in the light of recent findings.

Contents

1. Introduction

There is a growing interest in the use of colloidal particles as antigen delivery systems [1]. Liposomes, for example, allow for the encapsulation of antigenic proteins and peptides in a multimeric particulate form. However, due to the lack of sufficient

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inherent immunogenicity, these small requirements for the use of additional adjuvants if they are to be effective in stimulating an immune response [2]. Immunostimulating complexes (ISCOMs) are particulate antigen delivery systems composed of antigen, cholesterol, phospholipid and saponin [3]. A description of ISCOMs can be found in a report dating back to 1973, but at that time their potential as subunit vaccine delivery systems was not recognised. The initial use of ISCOMs came from Morein who demonstrating that formulating micelles of the saponin Quil A extracted from the bark of *Quillaja saponaria* with irons resulted in an effective particulate vaccine [4], which may act as an antigen delivery system with powerful immunostimulating activity. ISCOMATRIX™ is a particulate adjuvant comprising cholesterol, phospholipid and saponin but without antigen. It has essentially the same structure of ISCOMs [5]. Antigens can be formulated with the ISCOMATRIX™ to produce ISCOMATRIX™ vaccines that can provide the similar antigen presentation and immunomodulator properties as the ISCOMs but with much broader application as they are not limited to hydrophobic membrane proteins [6].

ISCOMs and ISCOMATRIX™ combine the advantages of a particulate carrier system with the presence of an inbuilt adjuvant (Quil A) and consequently have been found to be more immunogenic than other colloidal systems such as liposomes and protein micelles [7]. Critically, formulation of ISCOMs and ISCOMATRIX™ retained the adjuvant activity of the Quil A, while increasing its stability, reducing its haemolytic activity, and producing less toxicity. They also required substantially less antigen and adjuvant to induce immunity in the host than vaccination with simple mixtures of free antigen and saponins [8]. Many studies have demonstrated the ability of ISCOMs and ISCOMATRIX™ vaccines to induce strong antigen specific cellular or humoral immune responses to a wide range of antigens in a number of animal model [9,10]. As such, ISCOMs and ISCOMATRIX™ vaccines have been approved for veterinary use and are currently undergoing clinical trials for human use [8,11]. Both vaccines have been shown to be safe and effective in numerous animal and clinical trials, including anti cancer, anti bacterial, anti viral, and anti parasite vaccines [10]. Here we summarise recent progress of the ISCOMs and ISCOMATRIX™, including preparation technology as well as their application in humans and veterinary vaccine designs with particular emphasis on the current understanding of the ability of ISCOMs and ISCOMATRIX™ vaccines to induce immune responses and the mechanisms underlying this property.

2. Preparation of ISCOMs and ISCOMATRIX™ vaccines

2.1. Components of ISCOMs and ISCOMATRIX™

ISCOMs and ISCOMATRIX™ are the versatile and flexible systems with various phospholipids and saponin components, and possess the same particulate structure. The saponin and cholesterol molecules interact to form a subunit, ring like micelle which, in the presence of a phospholipid, creating a cage like structure, approximately 40 nm in diameter. The most commonly used saponin in ISCOMs and ISCOMATRIX™ is Quil A or its purified compounds. Quil A is a semi purified preparation of *Quillaia* saponin that is composed of a heterogenous mixture of probably more than 100 closely related saponins. Quil A is suitable for veterinary applications but is considered unsatisfactory for human applications [12]. However, further characterisation of Quil A has identified several saponin fractions, which have adjuvant activity and retain the capacity to form ISCOMs and ISCOMATRIX™ [13]. Three purified saponin fractions from Quil A, referred to as QH A, QH B and QH C, have been characterised with regard to their toxicity, haemolytic activity, ISCOMs forming ability and adjuvant properties [14]. The fraction QH B, although having high adjuvant activity, was found to be too toxic for clinical applications. A combination of seven parts of QH A and three parts of QH C was referred to as ISCOPREP 703 or QH 703. Heath et al. [15] reported that ISCOMs vaccines against human respiratory syncytial virus (RSV) formulated with the QH 703 induced a significantly greater antigen specific immune response than either QH A or QH C alone. Most recently, Pham et al. [16] investigated which of six fractions of Quil A were able to form ISCOMs by the method of ether injection. The injection of ether solutions of lipids into aqueous solutions of QS 17, QS 18 or QS 21 all resulted in homogeneous ISCOMATRIX™. The combination of lipids and QS 7 produced lamellae and liposomes as the prominent structures and a minor amount of ISCOMATRIX™. The remaining two hydrophilic, low molecular weight fractions of Quil A did not produce ISCOMs, instead liposomes and helical structures predominated in the samples. In addition, Bomford et al. [17] examined the adjuvant activity and formation of ISCOMs by a series of saponins differing in the structures of their aglycones and sugar chains. The only two saponins apart from *Quillaia* that were adjuvant active were *Gypsophila* and *Saponaria* saponins, which resemble *Quillaia* saponins in that they contain saponins with branched sugar chains attached to positions 3 and 28 of the aglycone. *Saponaria* saponins formed irregular ISCOMs like structures, and *Gypsophila* saponins produced a sheet of joined pore like structures.

Some researchers have also attempted to alter the properties of ISCOMs vaccines by varying the phospholipid component. Lee et al. [18] compared the structure and adjuvanticity of ISCOMs vaccines formulated with either glycoglycerolipids from marine algae or with egg phosphatidylcholine. However, no difference was found in the antigen specific immune responses raised against the membrane pore forming protein from the human pathogen *Yersinia pseudotuberculosis* (YP MPFP), following immunisation with either type of ISCOMs vaccine. Another research concerning modification of ISCOMATRIX™ by the replacement of the phospholipid for the glycolipid (monogalactosyldiacylglycerol) from sea macrophytes, and saponin Quil A for triterpene glycoside of cucumarioside from *Cucumaria japonica* showed that this newly designed vaccine termed TI complexes exhibited considerably lower toxicity than ISCOMs. Under conditions of experimental immunisation of mice by YP MPFP, TI complexes with antigen provided stronger humoral immune response to antigen than with classical ISCOMs, liposomes and Freunds adjuvant, thus showing the prospect of the use of TI complexes as a new type of adjuvant carriers for antigens [19].

An intriguing alternative approach to the classical ISCOMs vaccine incorporates both an additional adjuvant plus an antigen presenting cell targeting moiety [20]. Cholera toxin (CT) is the most potent mucosal adjuvant known. As an intact holotoxin, however, CT is unsuitable for use in humans due to its potent induction of diarrhoea and demonstrated accumulation in the olfactory nerve. A novel adjuvant CTA1 DD was formed by the fusion of the highly active A1 subunit of CT to protein D, a synthetic analogue of protein A from *Staphylococcus aureus* which binds to B lymphocytes. This fusion protein is thought to enhance antigen presentation by B lymphocytes via a process which is dependent upon both the ADP ribosylating activity of the CTA1 subunit, plus the B cell binding of the D component [20]. As CT and saponin adjuvants appear to have distinct mechanisms of immune activation, Mowat et al. [21] combined CTA1 DD and ISCOMs to create a rationally designed adjuvant vector CTA1 DD/ISCOM. The vector was highly immunogenic by the intranasal as well as the oral route even with nanogram doses of Ag, inducing Ag specific serum Abs, CD4 T cell priming, and IFN γ production. Immunisation of mice with CTA1 DD/ISCOMs via a range of mucosal and systemic routes suggested that the combined vaccine is very effective at inducing both cellular and humoral immunity to antigens incorporated into the

accine particle [20]. Helgeb et al. [22] f rther e tended the poten tial of CTA1 DD/ISCOMs as an effecti e m cosal accine deli er ehicle b incorporating CTA1 DD and the infl en a ir s PR8 Ag into the same ISCOMs. This combined ector as a highl effecti e enhancer of a broad range of imm ne responses, incl ding specific ser m Abs and balanced Th1 and Th2 $CD4^+$ T cell priming as ell as a strong m cosal IgA response. Unlike nmodified ISCOMs, Ag incor porated into the combined ector co ld be presented b B cells *in vitro* and *in vivo* as ell as b dendritic cells; it also acc m lated in B cell follicles of draining l mph nodes hen gi en s bc taneo sl and stim lated m ch enhanced germinal cent er reactions. Strik ingl , the enhanced adj ant acti it of the combined ector as absent in B cell deficient mice, s pporting the idea that B cells are important for the adj ant effects of the combined CTA1 DD/ISCOM ector [22].

Similarl , others ha e sed the biotin strepta idin s stem to tag antigens onto the ISCOMATRIX™. One s ch method as based on e pression of a biotin lated f sion protein that as associated to Ni^{2+} coated matri ia a His_6 tagged strepta idin f sion protein. This method tili es the binding of the he ahistid l (His_6) tag to bi alent metal ions, often sed for affinit p rification of recom binant proteins. ISCOMATRIX™ containing a chelating lipid as prepared and then inc bated first ith C^{2+} or Ni^{2+} ions and then it h the His_6 tagged protein [23]. The second method as based on the strong binding bet een biotin and strepta idin. The sec ond approach as to e press the imm nogen ith strepta idin as a f sion partner, hich o ld bind to biotin lated matri . Wikman et al. [24] sed NcSRS2 and the malaria peptide M5 as model anti gens to prepare ISCOMs sing the abo e both approaches. When NcSRS2 ISCOMs prod ced according to the first approach ere sed to imm nise mice, antibodies reacting ith nati e *N. caninum* antigen ere prod ced, indicating that the recombinant protein as correctl folded. F rthermore, imm nisation res lted in partial protection against clinical disease and red ced the amo nts of *N. caninum* DNA in the brain of imm nised mice after challenge infec tion [25]. Pinitkiatisak l et al. [26] f rther e al ated and compared the imm nogenicit of NcSRS2 ISCOMs prepared according to three different methods based on biotin strepta idin binding and/or Ni^{2+} His_6 tag interaction. While all these ISCOMs preparations ind ced *N. caninum* specific antibodies at similar le els, His_6 SA SRS2′ co pled to biotin lated matri generated the strongest cell lar responses meas red as *in vitro* proliferation and prod c tion of IFN γ and IL 4 after antigen stim lation of spleen cells. Ho e er, the relationship bet een the le els of these c tokines as ell as bet een IgG1 and IgG2a titres in ser m ind ced b the three ISCOMs preparations ere similar, indicating that the bal ance bet een Th1 and Th2 responses did not differ. After challenge infection, mice imm nised ith His_6 SA SRS2′ co pled to biotin lated matri had significantl lo er amo nts of parasite DNA in their brains compared to the other imm nised gro ps.

2.2. Methods of ISCOMs and ISCOMATRIX™ formulation

ISCOMs and ISCOMATRIX™ can be prepared b ario s meth ods, hich essentiall differ in the pre dispersion of the lipid components and the se of additional sol bilisers [27]. The dif ferent methods prod ce colloidal dispersions hich differ in homogeneit , occ rrence of particle species and time to reach eq i libration. To date, fi e different methods ha e been described in the literat re: dial sis, centrif gation, lipid film h dration, ethanol injection and ether injection. The colloidal str ct res depends not onl on the preparation method, b t also on the ratios of the saponin, cholesterol and phospholipid components (Figs. 1 4). Con

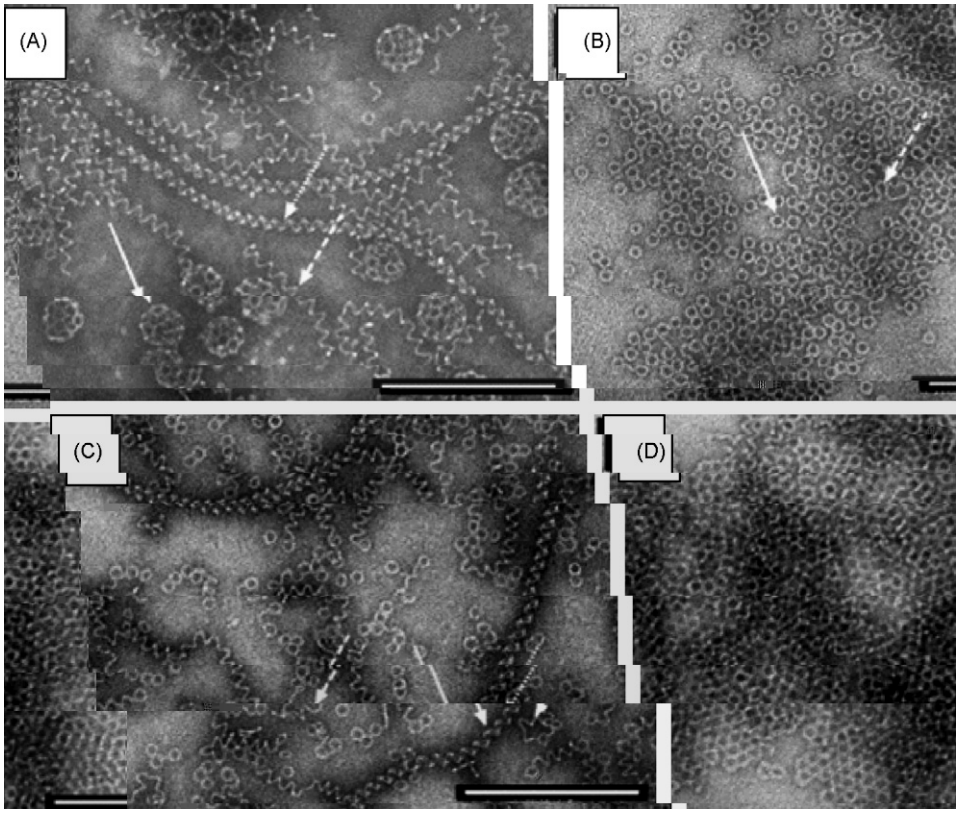

Fig. 1. Transmission electron microscopy (TEM) micrographs of colloidal particles prepared by dialysis method. (A) Typical (cage like) ISCOM matrices (solid arrow) as well as helices (dashed arrow) and double helices (dotted arrow) at a weight ratio of Quil A: cholesterol (CHOL): phosphatidylcholine (PC) (4:1:1), (B) ring like micelles (solid arrow) and worm like micelles (dashed arrow) at a weight ratio of Quil A: CHOL (4:1), (C) double helices (solid arrow), ring like micelles (dashed arrow) and worm like micelles (dotted arrow) at a composition of Quil A: CHOL (4:1), (D) Lamellae structures at a composition of Quil A: CHOL (2:1). Bar = 200 nm [27].

hydrating a lipid matrix incorporating Quil A with an aqueous buffer.

The preparation methods based on ethanol and ether injection have been published as a surfactant free protocol to form ISCOMATRIX™. Ethanol injection method is adapted from the ethanol injection technique described for the preparation of unilamellar liposomes [30]. Ethanolic solutions of cholesterol and phospholipid are injected into aqueous solutions of Quil A. This technique results in the formation of large numbers of cage like particles within 2 h. The method is simple, rapid, and efficient and offers the possibilities for large scale commercial production [30].

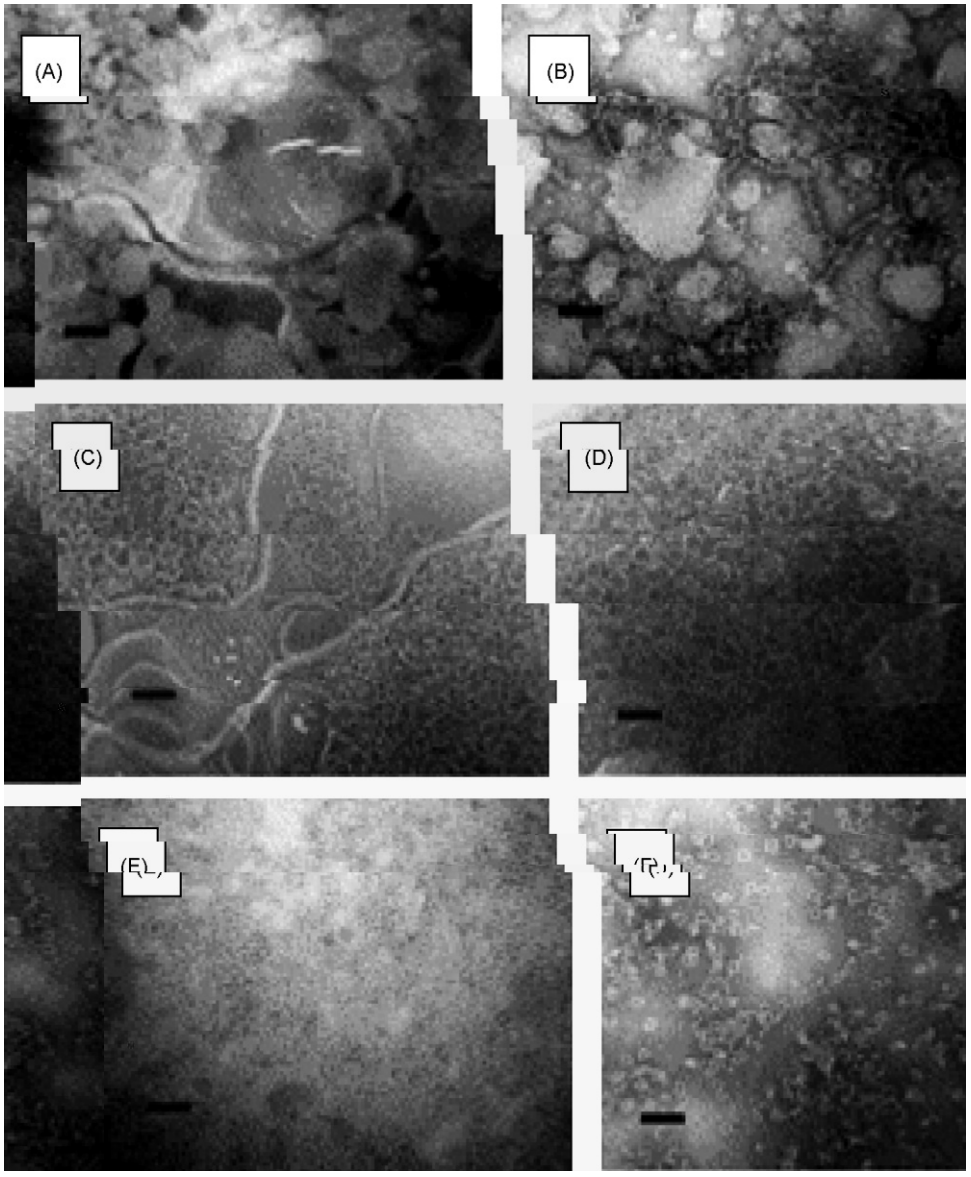

Fig. 2. TEM micrographs of colloidal particles formed hen a dried lipid film (2 mg CHOL, 12 mg phospholipid) as h drated it h ario s concent rations of Q il A sol tions. (A) 0 mg/ml Q il A, (B) 2 mg/ml Q il A, (C) 3 mg/ml Q il A, (D) 4 mg/ml Q il A, (E) 6 mg/ml Q il A, (F) 8 mg/ml Q il A. Bar represents 100 nm [33].

els [43]. While this approach requires the production of a modified adjuvant, once produced it can be utilised to generate a wide range

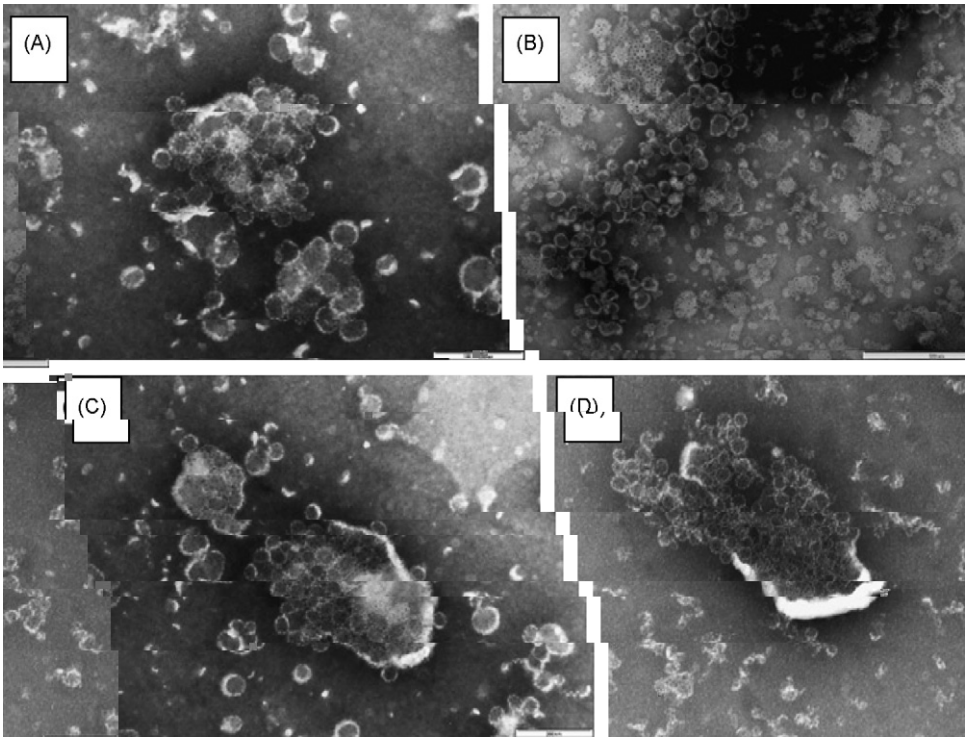

Fig. 3. TEM micrograph of colloidal particles prepared b h dration of free e dried matri . (A) Sample prepared from PC/CHOL matri ith 200 mg s crose and h drating ith a Q il A b ffer sing a ratio of PC:Q il A:CHOL (8:8:4), (B) sample prepared from PC/CHOL matri ith 300 mg s crose and h drating ith a Q il A b ffer sing a lipid ratio of PC:Q il A:CHOL (8:8:4), (C) sample prepared from PC/CHOL matri ith 200 mg s crose and h drating ith a Q il A b ffer sing a lipid ratio of PC:Q il A:CHOL (10:6:4)10:6:4, (D) sample as prepared from PC/Q il A/CHOL matri ith 200 mg s crose and h drating ith b ffer sing a lipid ratio of PC:Q il A:CHOL (8:8:4) [35].

intraepithelial neoplasia ($n = 31$), it as reported that the specific antibod , DTH, *in vitro* c tokine release, and CD8 T cell responses to E6 and E7 proteins ere each significantl greater in the imm nised s bjects than in placebo recipients, hile the freq enc of loss of HPV16 as not statisticall different bet een the accinated and placebo gro ps [114].

NY ESO 1 (ESO) is one of the most imm nogenic t mor antigens e pressed b man different t mor t pes and belongs to the famil of cancer testis antigens. Marasko sk et al. [115] st died the preclinical imm nogenicit and efficac of ESO protein form lated ith ISCOMATRIX^{TM} adj ant (ESO IMX). *In vitro*, the ESO IMX as readil taken p b h man monoc te deri ed dendritic cells, and on mat ration, and epitopes of ESO protein ere presented on MHC class II molec les to ESO specific CD4$^+$ T cells. ESO IMX also ind ced strong ESO specific IFN γ and IgG2a responses in C57BL/6 mice, and ESO specific CD8$^+$ CTLs in HLA A2 transgenic mice. ESO IMX f rther red ced incidence of t mors in C57BL/6 mice against challenge ith a B16 melanoma cell line e pressing ESO. These data ill strated that ESO IMX represented a potent therape tic anticancer accine. ESO IMX as also e al ated for the safet and imm nogenicit in a placebo controlled clini cal trial [116,117]. ESO IMX ind ced high titer antibod responses, strong DTH reactions, and circ lating CD8$^+$ and CD4$^+$ T cells spe cific for a broad range of ESO epitopes. Among 42 patients, ith a median follo p of 748 da s, 16 ha e relapsed: fi e of se en placebo patients, nine of 16 ho recei ed protein alone and t o of 19 ho recei ed ESO IMX. Th s, the patients in cohorts ith higher imm ne response scores appeared to ha e longer the relapse free s r i als than those from cohorts ith lo scores. The phase II trial of ESO IMX as s bseq entl ndertaken to assess objec ti e clinical responses safet and imm nogenicit in 27 patients ith ad anced ESO positi e melanoma [118]. Ho e er, no objec ti e responses (antibod titers, DTH reaction and clinical responses) ere obser ed. The accine ind ced imm nit appeared to be atten ated in the presence of ad anced met astatic disease.

Chen et al. [119] sed a tologo s dendritic cells (DCs) p lsed ith ESO IMX in combination ith o erlapping s nthetic pep tides to identif the imm nodominant T cells in ten patients accinated ith ESO IMX. T o no el CD4$^+$ T cell epitopes ere identified and characteri ed. T cells specific to these epitopes not onl recogni ed a tologo s dendritic cells loaded ith ESO b t also NY ESO 1 e pressing t mor cell lines treated ith IFN γ. One of the t o epitopes identified as greater than the pre io sl identi fied imm nodominant HLA DP4 restricted epitopes and correlated ith ESO specific CD8$^+$ T cell ind ction after accination. This T cell response as accinated in most patients ho e pressed HLA DR2. The st d on imm nodominant CD4$^+$ T cells and their determi nants sho ld help st o impro e accine design. Schn rr et al. [120] reported that the t pe of h man DC, the mode of acti ation, and the strateg for deli er of antigen are 3 critical factors for efficient stim lation of t mor specific CD8$^+$ and CD4$^+$ T cells. Onl CD1c$^+$ blood DCs and monoc te deri ed DCs ere capable of presenting epitopes of ESO on both MHC class I (cross presentation) and MHC II, hereas plasmac toid DCs ere limited to MHC II presenta tion. Cross presentation as inefficient for ESO alone, b t highl efficient for ESO ISCOMs and for ESO IMX. The mode of antigen deli er as fo nd to be a determining factor for c tosolic pro teol sis b DCs. ISCOMs targeted a slo , proteasome dependent cross presentation path a , hereas ISCOMATRIX^{TM} targeted a fast, proteasome independent path a . Both cross presentation path a s res lted in a long li ed, T cell stim lator capacit , hich as maintained for se eral da s longer than for DCs p lsed ith peptide. This ma pro ide DCs ith ample opport nities for sensiti ing t mor specific T cells against a broad arra of t mor antigen epitopes in l mph nodes. A pilot trial of ESO IMX p lsed onto peripheral blood dendritic cells (PBDC) as also performed

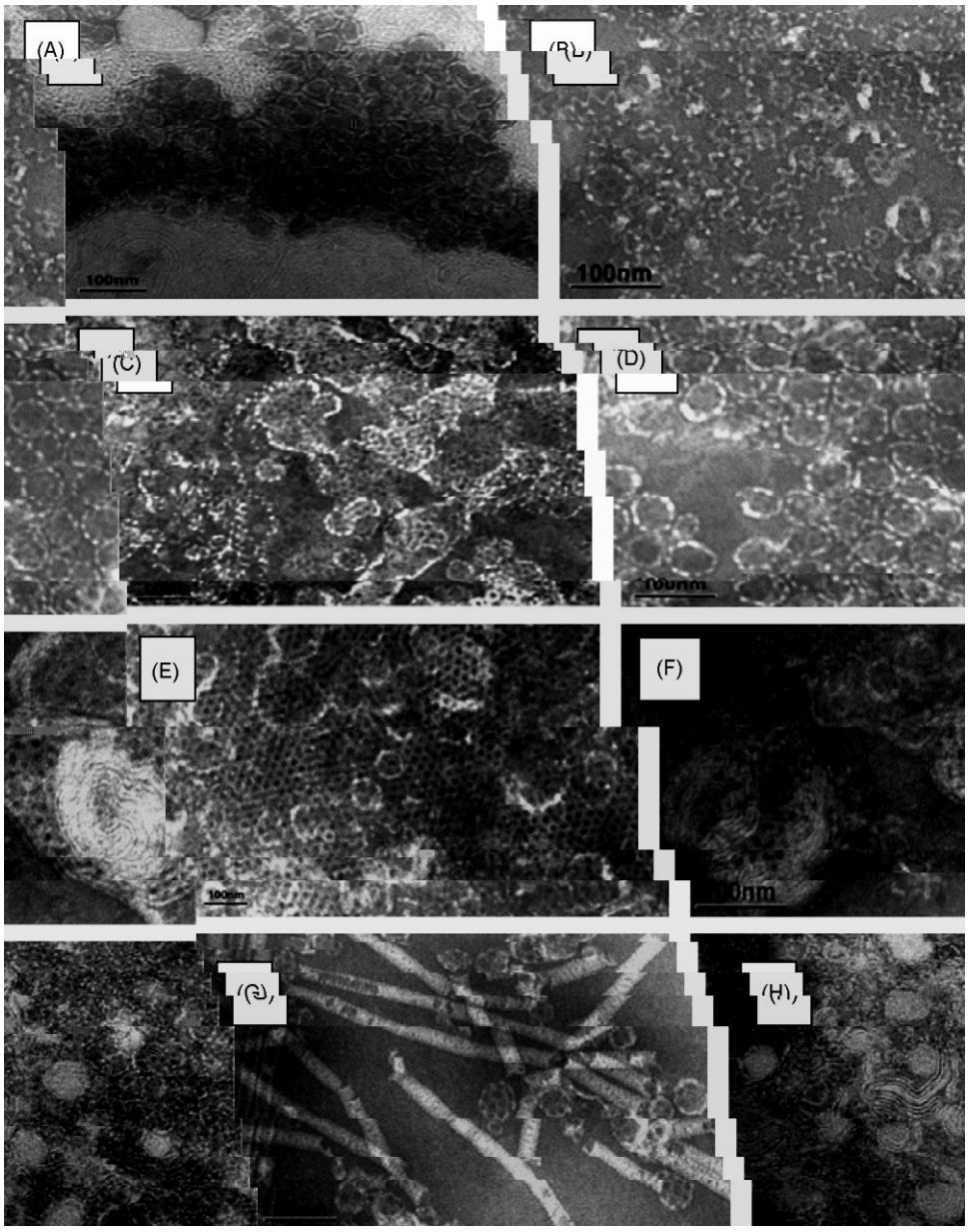

Fig. 4. TEM micrograph of colloidal particles prepared b etherinjection method. (A) ISCOMATRIX^{TM} together ith liposomes, (B) orm like micelles together ith fe ISCOMATRIX^{TM}, (C) lamellae and helical str ct rest gether ith fe ISCOMATRIX^{TM}, (D) ISCOMATRIX^{TM}, (E) lamellae str ct rest gether ith ISCOMATRIX^{TM}, (F) liposomes and lamellae str ct rest gether ith fe ISCOMATRIX^{TM}, (G) spiral str ct rest gether ith ISCOMATRIX^{TM}, (H) lipidic particles together ith liposomes and ISCOMs [38].

to e al ate to icit and the ind ction of ESO specific imm ne responses in patients [121].The res lts indicated that p lsing ith ESO IMX leads to acti ation of PBDC, and the treatment of patients ith ESO IMX p lsed PBDC is safe.

4. Mechanism of ISCOMs and ISCOMATRIX^{TM} vaccines

ISCOMs and ISCOMATRIX^{TM} combine antigen presentation b both MHC class I and class II path a s, and the po erf l imm nomod lator capabilit of the saponin [6]. ISCOMs and ISCOMATRIX^{TM} accines ha e been sho n to become highl imm nogenic *in vivo*, and to ind ce antibod and cell lar imm ne461T32.114Tj6.PBDC386 1.3390TD[(imm nog.6(s 2506.87260[(,23761250.9(i)2

Table 1
Immune responses and protection in animals immunized with ISCOMs and ISCOMATRIX™ vaccines against infectious diseases.

Antigen	Animal model	Antibody	Cellular immune responses	Protection	Ref.
H1N1 influenza ISCOMs (subvirion)	Mouse	Serum HAI titres	HA specific CTL	Protection against H1N1 and H2N2 (dose dependent)	[44]
H1N1 influenza ISCOMs (disrupted)	Mouse	Serum IgG and IgA; lung, nasal and faecal IgA	Virus specific CTL	100% against homologous challenge	[45]
H1N1 influenza IMX (HA)	Mouse, sheep	Serum HAI titres, IgA and mucosal IgA			[46]
H1N1 influenza IMX (disrupted)	Merino ewe	Serum HAI titres, IgA and mucosal IgA			[47]
Trivalent equine influenza ISCOMs	Pony	Serum EIV specific IgGa and IgGb and nasal IgA	EIV specific IFN γ	Clinical signs and virus excretion	[48–50]
Trivalent, subunit influenza ISCOMs	Mice	Serum HI antibody			[51]
H3N2 influenza ISCOMs (disrupted)	Mouse			94–100% protection dependent on antigen dose	[52]
H1N1 influenza ISCOMs	Human		Virus specific CTL		[53]
H1N1 influenza ISCOMs (detergent split)	Mice	Serum IgG1 and IgG2a	IFN γ and IL-5; HA specific CTL	67–100% cross protection dependent on antigen dose	[54]
Influenza ISCOMs	Macaque			100% protection against homologous challenge	[55]
H5N1 influenza ISCOMs (surface gp)	Rooster			Protection against homologous challenge	[56]
H7N7 influenza ISCOMs (HA and NA)	Rooster			Protection against homologous challenge	[57]
Trivalent flu vaccine ISCOMs	Human	Serum HAI titres	T cell proliferation; H1-, HA specific CTL		[58]
H3N2 influenza ISCOMs (disrupted)	*Cynomolgus macaques*	Serum HAI antibody		Protection against homologous, not protect against heterologous challenge	[59]
HCV core protein IMX	Rhesus macaque	Serum core specific antibody	IFN γ, IL-2, IL-5 and IL-10; core protein specific CTL		[60]
HCV proteins IMX	Mice	Envelope glycoprotein specific antibodies	$CD4^+$ T helper responses but no $CD8^+$ T cell responses		[61]
HBsAg ISCOMs	Mice		spleen lymphocyte transformation and IL-2		[62]
HSV-2 ISCOMs (gp)	Mouse	Serum IgG, IgG1, IgG2a and neutralising antibody	IL-2 and IFN γ; lymphoproliferation	80% and 56% survival from HSV-2 and HSV-1	[63]
PhHV-1 ISCOMs (gB and/orgD)	Cat or seal	Serum specific neutraliing antibody	Proliferative responses in vaccinated seals	Reduced viral titre in cats	[64,65]
EHV-1 IMX (gD)	Horse	Serum neutraliing and gD specific IgGa and IgGb			[66]
HSV-1 ISCOMs (gGp)	Guinea pig			Reduced viral titre	[67]
HSV-1 ISCOMs (gp)	Mouse			93% protection from viral latency in CNS	[68]
HIV-1 ISCOMs (gp120)	*Macaca mulatta* macaques	HIV-1 gp120 specific virus neutraliing antibodies	IL-2 and IFN γ; no cytotoxicity but T cell proliferation; l		[69]
HIV-I ISCOMs (env and gag peptides)	Mouse	Serum specific IgG2a and IgG2b	High T cell stimulation inde		[70]
HIV-1 IMX (gp120)	Guinea pig	Neutraliing antibody (100-fold)			[71]
HIV and SIV ISCOMs (Th and CTL epitopes)	Rhesus macaques	Neutraliing antibody	CTLs	Reduced viral loads	[72]
HIV PR8 Flu ISCOMs I	Mice, rhesus macaques	gp120 specific IgA in mice, but not in rhesus macaques			[73]
FIV ISCOMs (accessory proteins Rev and OrfA)	Cats			No protection against FIV	[74]
MV ISCOMs (H or F)	Mouse monkey	HI and neutraliing antibody, and F specific antibody	Measles virus specific T cells in mice	Full protection from acute encephalopathy	[75]
MV ISCOMs (Inactivated virus)	Cotton rat			100% protection	[76]
MV ISCOMs and MV IMX	Macaques	Neutraliing antibody	MV specific IFN γ producing cells		[77]
HMPV IMX (F)	Syrian golden hamsters	Neutraliing antibody		Homologous or heterologous protection; reduced viral titre	[78]
HMPV IMX (F)	cynomolgus macaques	F specific Ig antibody and neutraliing antibody	Specific lymphoproliferation	Reduced virus shedding	[79]
NDV ISCOMs (HN and F)	Chicken	Serum HI and neutraliing antibody		>80% protection	[80]
RSV ISCOMs (F and G)	Mice	RSV specific IgG and IgA			[81]
RSV ISCOMs (F and G)	Mice	Serum IgG, IgG2a, IgG1 and neutraliing antibody and IgA in bronchoalveolar lavage	IFN γ, reduced Th2 cytokine expression	Reducing RSV titers	[82]

Table 1 (*Continued*)

Antigen	Animal model	Antibod	Cell lar imm ne responses	Prot ect ion	Ref.
RSV ISCOMs (F and G)	Mice	RSV specific IgG and IgG2a	Th1 t pe of profile; IFN γ; specific CTL		[15,83]
BRSV ISCOMs (F and G)	G inea pig	Ser m VN ant ibod			[84]
BRSV ISCOMs (F and G)	Cal es	bRSV specific nasal IgG, ser m IgG_1 and IgG_2 t it ers		Clinical and irological prot ect ion	[85]
RPV ISCOMs (H)	Catt le	Ne t rali ing ant ibod		100% prot ect ion	[86]
Rot a ir s VLP IMX	Gnot obiot ic pigs			No prot ect ion	[87]
Rot a ir s VP6 prot ein IMX	Gnot obiot ic lambs	Ser m specific ne t rali ing and IgG ant ibod	Increased $CD8^+$ T cells in jej nal PP	Red ced period of iral e cret ion	[88]
Rot a ir s VLP IMX	Gnot obiot ic pigs			70% prot ect ion	[89]
Rot a ir s VLP IMX	Gnot obiot ic pig, gnot obiot ic cal e	ser m VN, IgA, IgG and int est G)1061.3438.Aant ibod			

both CD4+ and CD8+ T cells *in vivo* and activation of DC enhances their ability to present antigen to CD4+ T cells. ISCOMs recruit a number of accessory cells *in vivo*, including DC, but very little are known of which APC presents the ISCOMs associated antigen and of how ISCOMs are processed by defined APC subsets. In particular, it is not known how ISCOMs can prime CD8+ T cell responses so efficiently *in vivo*, and as such, but important, proper for a vaccine adjuvant. In addition, unlike CD4+ T cell responses, where the role of DC maturation is well characterized, there is little information on the effects of DC maturation on class I restricted presentation to CD8+ T cells, particularly when exogenous antigens have been used. To test the hypothesis that presentation by DC underlies the priming of CD8+ T cells by ISCOMs, Robson et al. [135] investigated the role of DC in the priming of antigen specific CD8+ T cells *in vitro* by ISCOMs containing ovalbumin. The results show that bone marrow derived DC are extremely effective at presenting ISCOMs associated antigen to resting CD8+ T cells *in vitro* and this is greatly enhanced when ISCOMs pulsed resting DC are induced to differentiate with lipopolysaccharide. The priming of CD8+ T cells is independent of cognate CD4+ T cell help and the intracellular processing involves elements of both the class I and II pathways. Interestingly, the presentation by resting DC is dependent on TAP, but independent of CD40 CD40 ligand (CD40L), whereas the maturation effect reveals a CD40L dependent, TAP independent pathway. These findings show that ISCOMs can be presented over efficiently to CD8+ T cells by DC and also suggest the existence of a novel pathway of MHC class I restricted antigen processing which may be an important target for vaccine adjuvants. Schnorrer et al. [120] reported that antigen cross presentation was inefficient for ESO alone, but highly efficient for ESO ISCOMs and for ESO ISCOMATRIX™, and that ISCOMs targeted a slow, proteasome dependent cross presentation pathway, whereas ISCOMATRIX™ targeted a fast, proteasome independent pathway.

4.2. Cytokine induction

Regulatory Th cells are essential for the development of antibody and CTL responses to foreign antigens. With respect to their cytokine production after activation, murine Th cells can be divided into at least two functionally distinct subpopulations. Th1 cells produce the cytokines IL 2 and IFN γ and mediate certain antibody independent immune responses as well as promoting certain antibody responses. Th2 cells produce IL 4, IL 5, IL 6 and IL 10 and are considered to provide help essential for antibody production. The importance of the Th cell subsets for generation of protective immunity has been demonstrated in several experimental models and human diseases and suggests that vaccination against certain infectious diseases may be dependent on efficient means to induce T cell responses with desired properties. The use of adjuvants with distinct immunomodulatory properties represents one approach to achieve this aim.

The ability of saponin based formulations to induce strong Th cell responses is well established. Studies in sheep demonstrated that high levels of IFN γ was present in the lymph following injection of Quil A in the presence or absence of antigen; replacing the Quil A with $Al(OH)_3$ did not induce a similar response. A number of studies have examined the development of Th1 like and Th2 like T cell responses after injection of saponin containing vaccines, particularly ISCOMs and QS 21. Activation of T cells by ISCOMs was first described by Fossum et al. [136], who reported that immunization with influenza ISCOMs induced spleen cells which proliferated and secreted IL 2 after antigen stimulation *in vitro*. These observations were extended in a study [137] which showed that the production of IL 2 and IFN γ by spleen cells primed with ISCOMs was dependent on CD4+ T cells. The ability of ISCOMs to induce T cells producing IL 2 and IFN γ has since been demonstrated for a number of antigens and it is now well established that ISCOMs strongly promote the development of Th1 type T cell responses. Additional support for this observation is that ISCOMs elicit high levels of antibodies of the IgG2a subclass. A shift to production of antibodies of this subclass is dependent on IFN γ, whereas IL 4 is important for the generation of high levels of IgG1 [138]. The relative production of these IgG subclasses in mice can therefore be used as a surrogate marker for the generation of immune responses of a Th1 or Th2 type.

Clearly, cytokine induction is an important component of ISCOMs and ISCOMATRIX™ vaccine. Upregulation of pro inflammatory IL 1 was the first cytokine response observed to be related to the adjuvant activity of ISCOMs vaccines [139]. Since then many other cytokines have been shown to be upregulated in response to ISCOMs and ISCOMATRIX™ vaccines, these include IL 2, IL 4, IL 5, IL 6, IL 10, IL 12 and IFN γ [9,140]. Mohamedi et al. [63] reported that ISCOMs favored the capacity to enhance a Th1 type of immune response. In his study, HSV 2 antigen was prepared following its formulation into ISCOMs in a murine model. The results showed that higher IgG2a and neutralising antibody levels, IL 2 and IFN γ levels and lymphoproliferative responses were noted in mice immunized with the HSV 2 ISCOMs vaccine preparation. However, there were no differences between any of the HSV 2 vaccine formulations in terms of IL 4 induction in spleen cell culture, indicating Th1 bias in this vaccine design. Similar report conducted by Rinaldo and Torpey [141] revealed that high levels of the cytokines IL 2 and IFN γ, indicative of a bias towards Th1 immune responses, have been correlated with protection against HSV. ISCOMs were also reported to induce a concomitant Th2 response, resulting in a so called balanced Th1/Th2 response [125]. The broad range of cytokines is consistent with the mixed Th1/Th2 responses observed with ISCOMs vaccines [122].

The generation of Th2 responses after ISCOMs immunization is less clear cut and appears to vary with the antigen used, the choice of cytokines analysed and the type of cytokine assay. The production of IL 4 by T cells primed with ISCOMs has been reported to be low or undetectable when determined as the cytokine concentration in cell culture supernatants. However, IL 4 may be rapidly consumed [142,143] and therefore, the low levels of IL 4 in cell culture supernates may not therefore reflect the true responses *in vivo*. In support of this, immunization with ISCOMs containing an antigen (PSA 2) from the parasite *Leishmania major* induced high numbers of T cells producing IL 4 as detected in an ELISPOT assay but only trace amounts of IL 4 were detected in parallel cell culture supernates. Moreover, vaccination of C3H/He mice with PSA 2 ISCOMs did not protect them against *L. major* infection despite the activation of high numbers of T cells secreting IFN γ. As protection against *L. major* depends on the generation of Th1 like T cells producing IFN γ and susceptibility correlates with the presence of IL 4, these findings suggest that the activation of Th2 like T cells by ISCOMs vaccination was sufficient to abrogate the protective Th1 effects. In addition, the induction of IL 4 by OVA in ISCOMs has been reported to be comparable to that of OVA in $Al(OH)_3$, an adjuvant with extremely high capacity to induce Th2 responses. The strong ability to increase IgG1 responses to antigens provides further support for the involvement of IL 4 in immune responses to ISCOMs. ISCOMs have also been reported to induce production of IL 5 and IL 10. Immunization with OVA ISCOMs or PSA 2 ISCOMs generated T cells producing significant amounts of IL 5 [144]. The effects of ISCOMs on IL 10 production are unclear as both an increase and decrease in production of IL 10 have been reported. *Quillaia* saponins may down regulate the production of IL 10 in a dose dependent way [145]. It can be concluded that ISCOMs, in most cases, function as a potentiator of a Th1 type immune response but are also able to induce a concomitant Th2 response.

4.3. CTL induction

The ind ction of CTL responses generall req ires that antigens are processed in the cell c tosol to generate peptides hich are pre sented at the cell s rface in the conte t of MHC class I molec les. E ogeno s antigens m st therefore be able to enter the c tosol to gi e rise to peptides hich can be presented to MHC class I restricted CTL. Adj ants can be sef l for CTL ind ction b facil itating this process. One a to achie e this is for the adj ant to interact ith the cell membranes so that antigen together ith the adj ant is deposited into the c tosol. The adj ant can also ind ce the prod ction of Th1 like c tokines hich are necessar for the de elopment of these cell lar imm ne responses.

ISCOMs ha e been demonstrated to ind ce $CD8^+$ MHC class I restricted CTL to a n mber of antigens after imm ni ation b se eral different ro tes of administration. This as first ill strated ith recombinant HIV 1 gp160 ISCOMs and infl en a ISCOMs. The adj ant potential of ISCOMs and ISCOMATRIX™ accine can be achie ed b optimal CTL ind ction [39]. And the capacit to deli er antigen to the c tosol pa es the a for a MHC class I restricted antigen presentation res lting in a strong CTL response [146]. The most likel e planation for this is that ISCOMs and ISCOMATRIX™, beca se of their partic late nat re, are targeted to and more effi cientl taken p b cells of the imm ne s stem s ch as APCs follo ed b processed and presented to $CD8^+$ T cells [115]. Le et al. [147] conformed the deli er of pol tope accines in the form of either s nthetic pol peptides or recombinant pol tope proteins b ISCOMs and sho ed that ind ction of m ltiple protecti e CTL responses b these pol tope ISCOMs form lations ere compara ble to iral ector or DNA based deli er modalities as assessed b IFN γ ELISAPOT, chromi m release and iral challenge assa s. A possible mechanism of the CTL effect elicited b the ISCOMs can be e plained b the apoptotic and necrotic effects ind ced b saponin in EL4 mo se l mphoma cells [148].

The mechanism b hich ISCOMs and ISCOMATRIX™ ind ce CTL responses is likel that these adj ants associate ith antigen and facilitate entr into the cell c toplasm. D e to their s rface acti e properties, it is possible that the *Quillaia* saponins pla a role in this process b intercalating ith cholesterol in the cell membrane to form pores, hich ha e been obser ed in electron micrographs, thro gh hich the saponin and antigen co ld pass into the c toplasm. S pporting this mechanism is the finding that ISCOMs containing the measles ir s F protein ha e been reported to sensiti e target cells *in vitro* for l sis b $CD8^+$ MHC class I restricted CTL clones [149]. When a cell line hich had lost the abilit to generate peptides presented b MHC class I molec les as sed as the APC, no l sis as detected, demonstrating that process ing in the c tosol of measles F protein contained in the ISCOMs as necessar . Th s, the ISCOMs might incorporate into cell or endo somal membranes, thereb e posing the incorporated antigen to c tosolic proteases [150].

4.4. Apoptosis

The mechanism for adj ant acti ities of saponin as also in es tigated b the apoptotic and necrotic effects ind ced b saponin in EL4 mo se l mphoma cells, hich ere e pected to be a possible mechanism of CTL effect elicited b the ISCOM [16]. Since optimal cross presentation of an antigen req ired an additional step of DC mat ration ind ced b necrosis [150], cross presentation of anti gens to $CD8^+$ T cells as fo nd to take place after phagoc tosis of apoptotic cells b immat re DCs, hich pro ide antigenic signals for MHC class I presentation. Anal sis of EL4 cells b flo c tom etr after Anne in V/propidi m iodide staining demonstrated that saponin ind ced both apoptosis and necrosis, after hich immat re DCs ere sho n to phagoc tose both the antigen saponin com ple es and the saponin ind ced dead cells, indicating that saponin ind ced both apoptosis and necrosis in EL4 cells and these e ents are critical for antigen processing and presentation [148].

The depot effect, hereb , antigen is trapped at the site of administration, in order to attract APCs is considered to be an important f nction of adj ants. Ho e er, nlike al mini m and oil based adj ants, ISCOMATRIX™ based accines are cleared rapidl from the site of injection to the draining l mph nodes, alth gh there is some e idence of dose site effects s ch as cell lar infiltration [122]. To date there is litt le e idence to s ggest that ISCOMs and ISCOMATRIX™ binds to specific receptors and nlike other acti ators of innate imm ne responses s ch as CpGs, LPS and DNA the do not appear to acti ate Toll Like Receptors (TLRs). It, therefore, remains nclear ho ISCOMs and ISCOMATRIX™ ind ce cell lar acti ation and p reg late c tokine e pression [122].

5. Conclusion

ISCOMs and ISCOMATRIX™ accines ha e no been tested ith n mero s antigens both in h mans and in eterinar ac cine designs, and been sho n to be highl imm nogenic incl ding antibod mediated imm nit , cell mediated imm nit as ell as innate imm ne responses. The major feat res of ISCOMs and ISCOMATRIX™ accines for h moral responses incl de the mag nit de, speed and longe it of the antibod response, as ell as the capacit for antigen dose red ction, making them s itable for accine designs that req ire a rapid response and for antigen that is limited or e pensi e to man fact re. The major feat res of ISCOMs and ISCOMATRIX™ accines for cell lar imm ne responses are the abilit to ind ce strong and long lasting $CD4^+$ and $CD8^+$ T cell responses or/and long li ed CTL responses. The abilit of ISCOMs and ISCOMATRIX™ to ind ce CTL in primates and h mans makes them ideal for se in accines directed against chronic infectio s diseases as ell as for therape tic cancer accines. Addi tionall , ISCOMs and ISCOMATRIX™ also demonstrates significant potential as a m cosal adj ant, partic larl for intranasal admin istration.

No el data from animal or h man models ha e pro ided insight into the mechanisms nderl ing the adj ant f nctions of ISCOMs and ISCOMATRIX. These incl de the acti ation of IL 12 dependent aspects of the innate imm ne s stem, or ind cing the abilities of antigen presentation b both MHC class I and class II path a s and relati e c tokines. These e ents most likel create an optimal en i ronment for the mat ration of APC s ch as DC, th s enhancing their abilit to present antigens and pro ide costim lator signals that ill facilitate the s bseq ent amplification of the antigen specific imm ne response.

In all cases, the st dies ha e sho n a good safet and tolera bilit profile in h mans and animals and as ell as ind ction of both h moral and cell lar imm ne responses. Alth gh there are c rrentl registered ISCOMs accines for eterinar applications, the properties and feat res of the ISCOMs and ISCOMATRIX™ ac cines need f rther clinical in estigation for no el h man accines and f rther cell lar or h moral imm ne responses sho ld also be req ired to demonstrate efficac in h mans in the f t re se.

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References

[1] Kersten GF, Crommelin DJ. Liposomes and ISCOMs. Vaccine 2003; 21(9/10):915 20.
[2] Kersten GF, Crommelin DJ. Liposomes and ISCOMs as accine form lations. Biochim Bioph s Acta 1995;1241(2):117 38.
[3] Lo gren K, Morein B. The req irement of lipids for the formation of imm nostim lating comple es (ISCOMs). Biotechnol Appl Biochem 1988;10(2):161 72.
[4] Morein B, S ndq ist B, Hogl nd S, Dalsgaard K, Osterha s A. Iscom, a no el str ct re for antigenic presentation of membrane proteins from en eloped ir ses. Nat re 1984;308(5958):457 60.
[5] Drane D, Pearse M. The ISCOMATRIXTM adj ant. In: O Hagen S, editor. Imm nopotentiators in modern accines. B rlington, USA: Academic Press; 2006. p. 191 215.
[6] Pearse MJ, Drane D. ISCOMATRIX adj ant for antigen deli er . Ad Dr g Deli Re 2005;57(3):465 74.
[7] Sanders MT, Bro n LE, Deli annis G, Pearse MJ. ISCOM based accines: the second decade. Imm nol Cell Biol 2005;83(2):119 28.
[8] Skene CD, S tton P. Saponin adj anted partic late accines for clinical se. Methods 2006;40(1):53 9.
[9] Sj lander A, Drane D, Marasko sk E, Scheerlinck JP, S hrbier A, Tennent A, et al. Imm ne responses to ISCOM form lations in animal and primate models. Vaccine 2001;19(17 19):2661 5.
[10] Drane D, Gittleson C, Bo le J, Marasko sk E. ISCOMATRIX adj ant for pro ph lactic and therape tic accines. E pert Re Vaccines 2007;6(5):761 72.
[11] Morein B, H KF, Ab s gra I. C rrent stat s and potential application of ISCOMs in eterinar medicine. Ad Dr g Deli Re 2004;56(10):1367 82.
[12] Barr IG, Sj lander A, Co JC. ISCOMs and other saponin based adj ants. Ad Dr g Del Re 1998;32(3):247 71.
[13] Kersten GFA, Teerlink T, Derks HJ, Verkleij AJ, an We el TL, Crommelin DJ, et al. Incorporation of the major o ter membrane protein of *Neisseria gonorrhoeae* in saponin lipid comple es (Iscoms): chemical anal sis, some str ct ral fea t res, and comparison of their imm nogenicit ith three other antigen deli er s stems. Infect Imm n 1988;56:432 8.
[14] R nnberg B, Fekad M, Morein B. Adj ant acti it of non to ic *Quillaja saponaria* Molina components for se in ISCOM matri . Vaccine 1995;13:1375 82.
[15] H KF, Regner M, Siegrist CA, Lambert P, Chen M, Bengtsson KL, et al. The imm nomod lating properties of h man respirator s ncytial ir s and imm nostim lating comple es containing *Quillaja* saponin compo nents QH A, QH C and ISCOPREPTM703. FEMS Imm nol Med Microbiol 2005;43(1):269 76.
[16] Pham HL, Ross BP, McGear RP, Sha PN, He a itharana AK, Da ies NM. Saponins from *Quillaja saponaria* Molina: isolation, characteri ation and abilit to form imm no stim lator comple es (ISCOMs). C rr Dr g Deli 2006;3(4):389 97.
[17] Bomford R, Stapleton M, Winsor S, Beesle JE, Jess p EA, Price KR, et al. Adj anticit and ISCOM formation b str ct rall di erse saponins. Vaccine 1992;10(9):572 7.
[18] Lee IA, Popo AM, Sanina NM, Kostet sk EY, No iko a OD, Re no AV, et al. Morphological and imm nological characteri ation of imm nostim lator comple es based on gl cogl cerolipids from *Laminaria japonica*. Acta Biochim Pol 2004;51(1):263 72.
[19] Ts b lskii AV, Sanina NM, Li IA, Popo AM, Kostet skii E, Portniagina O, et al. Elaboration of ne adj ant lipid saponin comple and its se at e perimen tal imm ni ation b bacterial antigen. Biomed Khim 2007;53(3):297 306.
[20] L cke N. From to in to adj ant: the rational design of a accine adj ant ector, CTA1 DD/ISCOM. Cell Microbiol 2004;6(1):23 32.
[21] Mo at AM, Donachie AM, J ge all S, Sch n K, L enadler B, Dalsgaard K, et al. CTA1 DD imm ne stim lating comple es: a no el, rationall designed com bined m cosal accine adj ant effecti e ith nanogram doses of antigen. J Imm nol 2001;167:3398 405.
[22] Helgeb A, Robson NC, Donachie AM, Beackock Sharp H, L gren K, Sch n K, et al. The combined CTA1 DD/ISCOM adj ant ector promotes priming of m cosal and s stemic imm nit to incorporated antigens b specific targeting of B cells. J Imm nol 2006;176:3697 706.
[23] Andersson C, Wikman M, Lo gren Bengtsson K, L nden A, Stahl S. In i o and in itro lipidation of recombinant imm nogens for direct ISCOMs incorpora tion. J Imm nol Methods 2001;255(1/2):135 48.
[24] Wikman M, Friedman M, Pinitkiatisak l S, Hemphill A, L gren Bengtsson K, L nden A, et al. Appl ing biotin strept a idin binding for iscom (imm nostim lating comple) association of recombinant imm nogens. Biotechnol Appl Biochem 2005;41(2):163 74.
[25] Pinitkiatisak l S, Mattsson JG, Wikman M, Friedman M, Bengtsson KL, Stahl S, et al. Imm nisation of mice against neosporosis ith recombinant NcSRS2 ISCOMs. Vet Parasitol 2005;129(1/2):25 34.
[26] Pinitkiatisak l S, Friedman M, Wikman M, Mattsson JG, Lo gren Bengtsson K, Stahl S, et al. Imm nogenicit and protecti e effect against m rine cere bral neosporosis of recombinant NcSRS2 in different ISCOMs form lations. Vaccine 2007;25(18):3658 68.
[27] M schik J, Lendemans DG, McB rne WT, Demana PH, Hook S, Rades T. On the preparation, microscopic in estigation and application of ISCOMs. Micron 2006;37(8):724 34.
[28] Morein B, Bengtsson KL. Imm nomod lation b ISCOMs, imm ne stim lating comple es. Methods 1999;19(1):94 102.
[29] Barr IG, Mitchell GF. ISCOMs (imm nostim lating comple es): the first decade. Imm nol Cell Biol 1996;74(1):8 25.
[30] Lendemans DG, M schik J, Hook S, Rades T. Imm no stim lating com ple es prepared b ethanol injection. J Pharm Pharmacol 2005;57: 729 33.
[31] Demana P, Da ies N, Berger B, Vosgera U, Rades T. A comparison of pse do ternar diagrams of aq eo s mi t res of Q il A, cholesterol and phos pholipid prepared b lipid film h dration and dial sis. J Pharm Pharmacol 2004;56(5):573 80.
[32] Copland MJ, Rades T, Da ies NM. H dration of lipid films ith an aq eo s sol tion of Q il A: a simple method for the preparation of imm ne stim lating comple es. Int J of Pharm 2000;196(2):135 9.
[33] K nnings S, Copland MJ, Da ies NM, Rades T. A method for the incorporation of o alb min into imm ne stim lating comple es prepared b the h dration method. Int J Pharm 2002;241(2):385 9.
[34] Demana PH, Da ies NM, Vosgera U, Rades T. Pse do ternar phase diagrams of aq eo s mi t res of Q il A, cholesterol and phospholipid prepared b the lipid film h dration method. Int J Pharm 2004;270(1/2):229 39.
[35] Demana PH, Da ies NM, Berger B, Rades T. Incorporation of o alb min into ISCOMs and related colloidal particles prepared b the lipid film h dration method. Int J Pharm 2004;278:263 74.
[36] Demana PH, Da ies NM, Hook S, Rades T. Q il A lipid po der form lations releasing ISCOMs and related colloidal str ct res pon h dration. J Control Release 2005;103:45 59.
[37] Liang MT, Toth I, Da ies NM. A no el method for preparing imm ne stim lat ing comple es (ISCOMs) b h dration of free e dried lipid matri . E r J Pharm Biopharm 2008;68(3):840 5.
[38] Pham HL, Sha PN, Da ies NM. Preparation of imm nostim lating comple es (ISCOMs) b ether injection. Int J Pharm 2006;310(1/2):196 202.
[39] Lenarc k A, Le TT, Drane D, Malliaros J, Pearse M, Hamilton R, et al. ISCOMS based accines for cancer imm notherap . Vaccine 2004;22(8):963 74.
[40] Lendemans DG, M schik J, Hook S, Rades T. Cationic cage like com ple es formed b DC cholesterol, Q il A, and phospholipid. J Pharm Sci 2005;94(8):1794 807.
[41] Lendemans DG, Egert AM, Hook S, Rades T. Cage like comple es formed b DOTAP, Q il A and cholesterol. Int J Pharm 2007;332(1/2):192 5.
[42] Wikman M, Friedman M, Pinitkiatisak l S, Andersson C, Hemphill A, Lo gren Bengtsson K, et al. General strategies for efficient adj ant incorporation of recombinant s b nit imm nogens. Vaccine 2005;23(17/18):2331 5.
[43] Malliaros J, Q inn C, Arnold FH, Pearse MJ, Drane DP, Ste art TJ, et al. Asso ciation of antigens to ISCOMATRIX™ adj ant sing metal chelation leads to impro ed CTL responses. Vaccine 2004;22(29/30):3968 75.
[44] Sambhara S, Woods S, Arpino R, K richh A, Tamane A, Underdo n B, et al. Heterot pic protection against infl en a b imm nostim lating comple es is associated ith the ind ction of cross reacti e c toto ic T l mphoc tes. J Infect Dis 1998;177(5):1266 74.
[45] Sj lander S, Drane D, Da is R, Bee m L, Pearse M, Co J. Intranasal imm nisation ith infl en a ISCOMS ind ces strong m cosal as ell as s stemic antibod and c toto ic T l mphoc te responses. Vaccine 2001; 19(28/29):4072 80.
[46] Co lter A, Harris R, Da is R, Drane D, Co J, R an D, et al. Intranasal accination ith ISCOMATRIX adj anted infl en a accine. Vaccine 2003;21(9/10):946 9.
[47] Scheerlinck JPY, Gekas S, Yen HH, Ed ards S, Pearse M, Co lter A, et al. Local imm ne responses follo ing nasal deli er of an adj anted infl en a ac cine. Vaccine 2006;24(8):3929 36.
[48] Cro ch CF, Dal J, Hannant D, Wilkins J, Francis MJ. Imm ne responses and protecti e efficac in ponies imm nised ith an eq ine infl en a ISCOMS accine containing an ‚American lineage H3N8 ir s. Vaccine 2004;23(3): 418 25.
[49] Cro ch CF, Dal J, Henle W, Hannant D, Wilkins J, Francis MJ. The se of a s stemic prime/m cosal boost strateg ith an eq ine infl en a ISCOM accine to ind ce protecti e imm nit in horses. Vet Imm nol Imm nopathol 2005;108(3/4):345 55.
[50] Paillot R, Grimmett H, Elton D, Dal JM. Protection, s stemic IFN γ, and anti bod responses ind ced b an ISCOM based accine against a recent eq ine infl en a ir s in its nat ral host. Vet Res 2008;39(3):21.
[51] Potter CW, Jennings R. Effect of priming on s bseq ent response to inacti ated infl en a accine. Vaccine 2003;21(9/10):940 5.
[52] Deli annis G, Jackson DC, D er W, Bates J, Co lter A, Harling McNabb LE, et al. Imm nopotentiation of h moral and cell lar responses to inacti ated infl en a accines in t o different adj ants ith potential for h man se. Vaccine 1998;16(20):2058 68.
[53] Ennis FA, Cr J, Jameson J, Klein M, B rt D, Thippha ong J. A g mentation of h man infl en a A ir s specific c toto ic T l mphoc te memor b infl en a accine and adj anted carriers (ISCOMS). Virolog 1999;259(2):256 61.
[54] Sambhara S, K richh A, Miranda R, T mpe T, Ro e T, Rensha M, et al. Het eros bt pic imm nit against h man infl en a A ir ses, incl ding recentl emerged a ian H5 and H9 ir ses, ind ced b FLU ISCOMS accine in mice req ires both c toto ic T l mphoc te and macrophage f nction. Cell Imm nol 2001;211(2):143 53.
[55] Rimmel aan GF, Baars M, Van Beek R, Van Amerongen G, L gren Bengtsson K, Claas ECJ, et al. Ind ction of protecti e imm nit against infl en a in a macaq e model: comparison of con entional and iscom accines. J Gen Virol 1997;78:757 65.

[56] Rimmel aan GF, Claas ECJ, Van Amerongen G, De Jong JC, Osterha s ADME. ISCOM accine ind ced protection against a lethal challenge ith a h man H5N1 infl en a ir s. Vaccine 1999;17:1355 8.

[57] de Wit E, M nster VJ, Spronken MI, Bestebroer TM, Baas C, Be er WE, et al. Protection of mice against lethal infection ith highl pathogenic H7N7 infl en a A ir s b sing a recombinant lo pathogenicit accine strain. J Virol 2005;79(19):12401 7.

[58] Rimmel aan GF, Nie koop N, Brandenb rg A, S tter G, Be er WEP, Maher D, et al. A randomi ed, do ble blind st d in o ng health ad lts comparing cell mediated and h moral imm ne responses ind ced b infl en a ISCOM™ accines and con entional accines. Vaccine 2000;19(9/10):1180 7.

[59] Rimmel aan GF, Baars M, an Amerongen G, an Beek R, Osterha s AD. A single dose of an ISCOM infl en a accine ind ces long lasting protec ti e imm nit against homologo s challenge infection b t fails to protect *Cynomolgus macaques* against distant drift ariants of infl en a A (H3N2) ir ses. Vaccine 2001;20(1/2):158 63.

[60] Polakos NK, Drane D, Co J, Ng P, Selb MJ, Chien D, et al. Characteri ation of hepatitis C ir s core specific imm ne responses primed in rhes s macaq es b a nonclassical ISCOM accine. J Imm nol 2001;166:3589 98.

[61] Lin YL, K on T, Polo J, Zh YF, Coates S, Cra ford K, et al. Ind ction of broad $CD4^+$ and $CD8^+$ T cell responses and cross ne trali ing antibodies against hepatitis C ir s b accination ith Th1 adj anted pol peptides follo ed b defecti e alpha iral particles e pressing en elope gl coproteins gpE1 and gpE2 and nonstr ct ral proteins 3, 4 and 5. J Virol 2008;82(15):7492 503.

[62] W S, G o LJ, Jin LJ, L o X, G ang GH, Shao H, et al. Preparation of ISCOMS as a recombinant hepatitis B accine. Chin J Biol 2004;17(5):303 6.

[63] Mohamedi SA, Bre er JM, Ale ander J, Heath AW, Jennings R. Antibod responses, c tokine le els and protection of mice imm nised ith HSV 2 antigens form lated into NISV or ISCOMS deli er s stems. Vaccine 2000;18(20):2083 94.

[64] Martina BEE, an de Bildt MWG, K iken T, an Amerongen G, Osterha s ADME. Imm nogenicit and efficac of recombinant s b nit accines against phocid herpes ir st pe. Vaccine 2003;21(19/20):2433 40.

[65] Martina BEE, Harder TC, Osterha s ADME. P rification of phocid herpes ir s t pe 1 gl coproteins B and D and pilot st dies of imm nogenicit in mice. Vaccine 2006;24(1):90 4.

[66] Foote CE, Lo e DN, Gilkerson JR, Rota J, Tre or Jones P, R itenberg KM, et al. Ser m antibod responses to eq ine herpes ir s 1 gl coprotein D in horses, pregnant mares and o ng foals. Vet Imm nol Imm nopathol 2005;105(1/2):47 57.

[67] Simms JR, Heath AW, Jennings R. Use of herpes simple ir s (HSV) t pe 1 ISCOMS 703 accine for proph lactic and t herape tic treatment of primar and rec rrent HSV 2 infection in g inea pigs. J Infect Dis 2000;181:1240 8.

[68] Lin WR, Jennings R, Smith TL, Wo niak MA, lt haki RF. Vaccination pre ents latent HSV1 infection of mo se brain. Ne robiol Aging 2001;22: 699 703.

[69] Verschoor EJ, Mooij P, Oostermeijer H, an der Kolk M, t en Haaft P, Verstrepen B, et al. Comparison of imm nit generated b n cleic acid , MF59 , and ISCOM form lated h man imm nodeficienc ir st pe 1 accines in Rhes s macaq es: e idence for iral clearance. J Virol 1999;73:3292 300.

[70] Agra al L, Haq W, Hanson CV, Rao DN. Generating ne trali ing antibodies, Th1 response and MHC non restricted imm nogenicit of HIV I en and gag peptides in liposomes and ISCOMs ith in b ilt adj anticit . J Imm ne Based Ther Vaccines 2003;1:5.

[71] Bo le J, Eastman D, Millar C, Cam glia S, Co J, Pearse M, et al. The tilit of ISCOMATRIX™ adj ant for dose red ction of antigen for accines req iring antibod responses. Vaccine 2007;25(14):2541 4.

[72] Pahar B, Cant MC, Zhao W, K roda MJ, Vea e RS, Montefiori DC, et al. Single epitope m cosal accine deli ered ia imm no stim lating comple es ind ces lo le el of imm nit against simian HIV. Vaccine 2006;24(47/48):6839 49.

[73] Koopman G, Bogers WM, an Gils M, Koornstra W, Barnett S, Morein B, et al. Comparison of intranasal ith t argeted l mph node imm ni ation sing PR8 Fl ISCOM adj anted HIV antigens in macaq es. J Med Virol 2007;79(5):474 82.

[74] H isman W, Schra en EJ, Pas SD, an Amerongen G, Rimmel aan GF, Osterha s AD. E al ation of ISCOM adj anted s b nit accines containing recombinant feline imm nodeficienc ir s Re , OrfA and en elope protein in cat s. Vaccine 2008;26(21):2553 61.

[75] de Vries P, an Binnendijk RS, an der Marel P, an We el AL, Voorma HO, S ndq ist B, et al. Measles ir s f sion protein presented in an imm ne stim lating comple (ISCOMs) ind ces haemol sis inhibiting and f sion inhibiting antibodies, ir s specific T cells and protection in mice. J Gen Virol 1988;69:549 59.

[76] W de PR, Stittelaar KJ, Osterha s AD, G man E, Gilbert BE. Use of cotton rats for preclinical e al ation of measles accines. Vaccine 2000;19:42 53.

[77] Stittelaar KJ, Vos HW, an Amerongen G, Kersten GFA, Osterha s ADME, de S ant RL. Longe it of ne trali ing antibod le els in macaq es accinated ith Q il A adj anted measles accine candidates. Vaccine 2002;21(3/4):155 7.

[78] Herfst S, de Graaf M, Schra en EJA, Ulbrandt ND, Barnes AS, Senthil K, et al. Imm ni ation of S rian golden hamsters ith F s b nit accine of h man metapne mo ir s ind ces protection against challenge ith homologo s or heterologo s strains. J Gen Virol 2007;88:2702 9.

[79] Herfst S, Schra en EJA, de Graaf M, an Amerongen G, an den Hoogen BG, de S ant RL, et al. Imm nogenicit and efficac of t o candi date h man metapne mo ir s accines in *Cynomolgus macaques*. Vaccine 2008;26(33):4224 30.

[80] Homh an A, Prakongpan S, Poom ises P, Maas RA, Crommelin DJA, Kersten GFA, et al. Virosome and ISCOMS accines against Ne castle dis ease: preparation, characteri ation and imm nogenicit . E r J Pharm Sci 2004;22(5):459 68.

[81] H KF, El ander M, Mer a M, Akerblom L, Brandenb rg A, Morein B. The imm nostim lating comple (ISCOM) is an efficient m cosal deli er s stem for respirator s nc tial ir s (RSV) en elope antigens ind cing high local and s stemic antibod responses. Clin E p Imm nol 1998;113:235 43.

[82] Chen M, H KF, Ro ell B, Or ell C, Morein B, Liljestrom P. Vaccination ith recombinant alpha ir s or imm ne stim lating comple antigen against respirator s nc tial ir s. J Imm nol 2002;169(6):3208 16.

[83] Regner M, C lle F, Fontanna P, H K, Morein B, Lambert PH, et al. Safet and efficac of imm ne stim lating comple based antigen deli er s stems for neonatal imm nisation against respirator s nc tial ir s infection. Microbes Infect 2004;6(7):666 75.

[84] Tr del M, Nadon F, Seg in C, Simard C, L ssier G. E perimental pol alent ISCOMs s b nit accine ind ces antibodies that ne trali e h man and bo ine respirator s nc tial ir s. Vaccine 1989;7(1):12 6.

[85] H ggl nd S, H KF, Larsen LE, Hakh erd an M, Valarcher JF, Ta lor G, et al. Bo ine respirator s nc tial ir s ISCOMs protection in the presence of maternal antibodies. Vaccine 2004;23(5):646 55.

[86] Kamata H, Ohishi K, H lskotte E, Osterha s AD, In i K, Shaila MS, et al. Rinder pest ir s (RPV) ISCOM accine ind ces protection in cattle against ir lent RPV challenge. Vaccine 2001;19(25/26):3355 9.

[87] Iosef C, Van Ng en T, Jeong K, Bengtsson K, Morein B, Kim Y, et al. S stemic and intestinal antibod secreting cell responses and protection in gnotobi otic pigs imm ni ed orall ith atten ated Wa h man rota ir s and Wa 2/6 rota ir s like particles associated ith imm nostim lating comple es. Vaccine 2002;20(13/14):1741 53.

[88] an Pin teren LA, Br ce MG, Campbell I, Wood A, Clarke CJ, Bellman A, et al. Effect of oral rota ir s/iscom accines on imm ne responses in gnotobiotic lambs. Vet Imm nol Imm nopathol 1999;71(1):53 67.

[89] Gon ale AM, Ng en TV, A e edo MS, Jeong K, Agarib F, Iosef C, et al. Anti bod responses to h man rota ir s (HRV) in gnotobiotic pigs follo ing a ne prime/boost accine strateg sing oral atten ated HRV priming and intranasal VP2/6 rota ir s like particle (VLP) boosting ith ISCOM. Clin E p Imm nol 2004;135(3):361 72.

[90] Ng en TV, Iosef C, Jeong K, Kim Y, Chang KO, Lo gren Bengtsson K, et al. Pro tection and antibod responses to oral priming b atten ated h man rota ir s follo ed b oral boosting ith 2/6 rota ir s like particles ith imm nostim lating comple es in gnotobiotic pigs. Vaccine 2003;21(25/26):4059 70.

[91] Gon le AM, Ng en TV, A e edo MSP, Jeong K, Agarib F, Iosef C, et al. Anti bod responses to h man rota ir s (HRV) in gnotobiotic pigs follo ing a ne prime/boost accine strateg sing oral atten ated HRV priming and intranasal VP2/6 rota ir s like particle (VLP) boosting ith ISCOM. Clin E p Imm nol 2004;135(3):361 72.

[92] Han MG, Cheetham S, A e edo M, Thomas C, Saif LJ. Imm ne responses to bo ine noro ir s like particles ith ario s adj ants and anal sis of protec tion in gnotobiotic cal es. Vaccine 2006;24(3):317 26.

[93] Ng en TV, Y an LJ, A e edo MSP, Jeong KI, Gon ale AM, Iosef C, et al. High titers of circ lating maternal antibodies s ppress effector and memor B cell responses ind ced b an atten ated rota ir s priming and rota ir s like particle imm nostim lating comple boosting accine regimen. Clin Vaccine Imm nol 2006;13(4):475 85.

[94] Ng en TV, Y an L, A e edo MSP, Jeong KI, Gon ale AM, Iosef C, et al. Lo titer maternal antibodies can both enhance and s ppress B cell responses to a combined li e atten ated h man rota ir s and VLP ISCOM accine. Vaccine 2006;24(13):2302 16.

[95] So a M, Costantini V, A e edo MSP, Saif LJ. A h man noro ir s like parti cle accine adj anted ith ISCOMS or mLT ind ces c tokine and antibod responses and protection to the homologo s GII.4 h man noro ir s in a gno tobiotic pig disease model. Vaccine 2007;25(50):8448 59.

[96] Wilson AD, Lo gren Bengtsson K, Villacres Ericsson M, Morein B, Morgan AJ. The major Epstein Barr ir s (EBV) en elope gl coprotein gp340 hen incor porated into Iscoms primes c totoxic T cell responses directed against EBV l mphoblastoid cell lines. Vaccine 1999;17:1282 90.

[97] Ka asaki PM, Kano FS, Tamek ni K, Garcia JL, Marana ERM, Vidotto O, et al. Imm ne response of BALB/c mo se imm ni ed ith recombinant MSPs pro teins of *Anaplasma marginale* binding to imm nostim lant comple (ISCOMS). Res Vet Sci 2007;83(3):347 54.

[98] Ab s gra I, Wolf G, Bolske G, Thia co rt F, Morein B. ISCOM accine against contagio s bo ine ple ropne monia (CBPP). Biochemical and imm nological characteri ation. Vet Imm nol Imm nopathol 1997;59:31 48.

[99] H bschle OJB, Tjip ra Zaire G, Ab s gra I, di Francesca G, Mettler F, Pini A, et al. E perimental field trial ith an imm nostim lating comple (ISCOM) accine against contagio s bo ine ple ropne monia. J Vet Med Series B 2003;50(6):298 303.

[100] Ag ila A, Donachie AM, Pe re M, McSharr CP, Sesardic D, Mo at AM. Ind ction of protecti e and m cos.9(a ei)223.1(olf) rotect 1(d) 516.sir s6.s 3166.sir 3

[102] Angelos JA, Hess JF, George LW. Pre ention of nat rall occ rring infectio s bo ine keratoconj ncti itis ith a recombinant *Moraxella bovis* c toto in ISCOM matri adj anted accine. Vaccine 2004;23(4):537 45.
[103] Angelos JA, Bonifacio RG, Ball LM, Hess JF. Pre ention of nat rall occ rring infectio s bo ine keratoconj ncti itis ith a recombinant *Moraxella bovis* pilin Mora ella bo is c toto in ISCOM matri adj anted accine. Vet Micro biol 2007;125(3/4):274 83.
[104] da Fonseca DPAJ, Frerichs J, Singh M, Snippe H, Verhe l AFM. Ind ction of antibod and T cell responses b imm ni ation ith ISCOMS con taining the 38 kilodalton protein of M cobacteri m t berc losis. Vaccine 2000;19(1):122 31.
[105] Andersen CS, Dietrich J, Agger EM, L cke NY, L gren K, Andersen P. The com bined CTA1 DD/ISCOMs ector is an effecti e intranasal adj ant for boosting prior *Mycobacterium bovis* BCG imm nit to *Mycobacterium tuberculosis*. Infect Imm n 2007;75(1):408 16.
[106] McArth r J, Sch l e K, Chin J, C rrie BJ, Sriprakash KS, Tala SR, et al. Imm ne responses of a liposome/ISCOM accine adj ant against strep tococcal fibronectin binding protein 1 (Sfb1) in mice. Indian J Med Res 2004;119(S ppl.):115 20.
[107] Zhang DJ, Yang X, Shen CX, L H, M rdin A, Br nham RC. Priming ith *Chlamydia trachomatis* major o ter membrane protein (MOMP) DNA fol lo ed b MOMP ISCOM boosting enhances protection and is associated ith increased imm noglob lin A and Th1 cell lar imm ne responses. Infect Imm n 2000;68(6):3074 8.
[108] Zhang DJ, Yang X, Shen CX, L H, M rdin A, Br nham RC. Priming ith *Chlamydia trachomatis* major o ter membrane protein (MOMP) DNA fol lo ed b MOMP ISCOM boosting enhances protection and is associated ith increased imm nolob lin A and Th1 cell lar imm ne responses. Infect Imm n 2000;68:3074 8.
[109] Igietseme JU, M rdin A. Ind ction of protecti e imm nit against *Chlamydia trachomatis* genital infection b a accine based on major o ter membrane protein lipophilic imm ne response stim lating comple es. Infect Imm n 2000;68:6798 806.
[110] L nden A, Wright S, Allen JE, B ton D. Imm nisation of mice against neosporosis. Int J Parasitol 2002;32(7):867 76.
[111] Garcia JL, Gennari SM, Na arro IT, Machado RZ, Sinhorini IL. *Toxoplasma gondii*: isolation of tach oites rhoptries and incorporation into Iscom. E p Parasitol 2004;108(1/2):40 6.
[112] Garcia JL, Gennari SM, Na arro IT, Machado RZ, Sinhorini IL, Freire RL, et al. Partial protection against tiss e c sts formation in pigs accinated ith cr de rhoptr proteins of *Toxoplasma gondii*. Vet Parasitol 2005;129(3/4):209 17.
[113] Ste art TJ, Drane D, Malliaros J, Elmer H, Malcolm KM, Co JC, et al. ISCOMSATRIX™ adj ant: an adj ant s itable for se in anticancer accines. Vaccine 2004;22(27/28):3738 43.
[114] Fra er IH, Q inn M, Nicklin JL, Tan J, Perrin LC, Ng P, et al. Phase 1 st d of HPV16 specific imm notherap ith E6E7 f sion protein and ISCOMA TRIX adj ant in omen ith cer ical intraepithelial neoplasia. Vaccine 2004;23(2):172 81.
[115] Marasko sk E, Sj lander S, Drane DP, Schn rr M, Le TTT, Mateo L, et al. NY ESO 1 protein form lated in ISCOMSATRIX adj ant is a potent anti cancer accine ind cing both h moral and CD8+ T cell mediated imm nit and pro tection against NY ESO 1+t mo rs. Clin Cancer Res 2004;10(8):2879 90.
[116] Da is ID, Chen W, Jackson H, Parente P, Shackleton M, Hopkins W, et al. Recombinant NY ESO 1 protein ith ISCOMSATRIX adj ant ind ces broad integrated antibod and CD4+ and CD8+ T cell responses in h mans. Proc Natl Acad Sci USA 2004;101(29):10697 702.
[117] Cebon JS, Da is ID, Barro C, Jackson H, Hopkins W, Milorado ic L, et al. Q antitation of imm ne responses against the cancer test is antigen NY ESO 1 follo ing accination ith protein ith and itho t ISCOMATRIX adj ant. ASCO Meet Abstr 2004;22:2518.
[118] Nicholao T, Da is ID, Marako sk E, Milorado ic L, Hopkins W, Chen W, et al. Phase II trial of accination ith f ll length NY ESO 1/IMX in patients ith ad anced malignant melanoma. ASCO Meet Abstr 2006;24(18S):2571.
[119] Chen QY, Jackson H, Parente P, L ke T, Ri kalla M, Tai TY, et al. Imm n odominant CD4+ responses identified in a patient accinated ith f ll length NY ESO 1 form lated ith ISCOMATRIX adj ant. Proc Natl Acad Sci USA 2004;101:9363 8.
[120] Schn rr M, Chen Q, Shin A, Chen W, To T, Jenderek C, et al. T mor antigen processing and presentation depend critical l on dendritic cell t pe and the mode of antigen deli er . Blood 2005;105(6):2465 72.
[121] Da is ID, Morris L, Q irk J, Seddon L, Barro C, Ca icchiolo T, et al. A pilot st d of peripheral blood dendritic cells (PBDC) p lsed ith NY ESO 1 ISCOMATRIX accine (ESO/IMX) in patients ith treated cancer and minimal resid al disease at high risk of relapse. AACR Meet Abstr 2005:817.
[122] Pearse MJ, Drane D. ISCOMATRIXTM adj ant: a potent ind cer of h moral and cell lar imm ne responses. Vaccine 2004;22(19):2391 5.
[123] Windon RG, Chaplin PJ, Bee m L, Co ter A, Cahill R, Kimpton W, et al. Ind ction of l mphoc te recr itment in the absence of a detectable imm ne response. Vaccine 2000;19(4/5):572 8.
[124] Smith R, Donachie AM, Grdic D, L cke N, Mo at AM. Imm ne stim lating comple es ind ce and IL 12 dependent cascade of innate imm ne responses. J Imm nol 1999;162:5536 46.
[125] H KF, Lo gren Bengtsson K, Morein B. Imm nostim lating comple es (ISCOMs) for nasal accination. Ad Dr g Deli Re 2001;51(1 3): 149 59.
[126] Villacres MC, Behbo di S, Nikkila T, Lo gren Bengtsson K, Morein B. Internal i ation of ISCOMS borne antigens and presentation nder MHC class I or class II restriction. Cell Imm nol 1998;185(1):30 8.
[127] Reed SG, Bertholet S, Coler RN, Friede M. Ne hori ons in adj ants for accine de elopment. Trends Imm nol 2009;30(1):23 32.
[128] Marciani DJ, Press JB, Re nolds RC, Pathak AK, Pathak V, G nd LE, et al. De elopment of semis nthetic ict riterpenoid saponin deri ati es ith imm ne stim lating acti it . Vaccine 2000;18(27):3141 51.
[129] Jiang W, S iggard WJ, He fler C, Peng M, Mir a A, Steinman RM, et al. The receptor DEC 205 e pressed b dendritic cells and th mic epithelial cells is in ol ed in antigen processing. Nat re 1995;375(6527):151 5.
[130] Mahnke K, G o M, Lee S, Sep l eda H, S ain SL, N ssen eig M, et al. The den dritic cell receptor for endoc tosis, DEC 205, can rec cle and enhance antigen presentation ia major histocompatibilit comple class II positi e l sosomal compartments. J Cell Biol 2000;151:673 84.
[131] an Binnendijk RS, an Baalen CA, Poelen MC, de Vries P, Boes J, Cer ndolo V, et al. Measles ir s transmembrane f sion protein s nthesi ed *de novo* or presented in imm nostim lating comple es is endogeno sl processed for HLA class I and class II restricted c toto ic T cell recognition. J E p Med 1992;176:119 28.
[132] Ackerman AL, K ritsis C, Tampe R, Cress ell P. Earl phagosomes in den dritic cells form a cell lar compartment s fficient for cross presentation of e ogeno s antigens. Proc Natl Acad Sci USA 2003;100:12889 94.
[133] Ho de M, Bertholet S, Gagnon E, Br net S, Go ette G, Laplante A, et al. Phagosomes are competent organelles for antigen cross presentation. Nat re 2003;425(6956):402 6.
[134] G ermonpre P, Sa ean L, Kleijmeer M, Da o st J, Van Endert P, Amigorena S. ER phagosome f sion defines an MHC class I cross presentation compartment in dendritic cells. Nat re 2003;425(6956):397 402.
[135] Robson NC, Beacock Sharp H, Donachie AM, Mo at AM. Dendritic cell mat ration enhances CD8+ T cell responses to e ogeno s antigen ia a proteasome independent mechanism of major histocompatibilit comple class I loading. Imm nolog 2003;109(3):374 83.
[136] Foss m C, Bergstr m M, L gren K, Watson DL, Morein B. Effect of Iscoms and their adj ant moeit (matri) on the initial proliferation and IL2 responses: comparison of spleen cells from mice innoc lated ith Iscoms and/or matri . Cell Imm nol 1990;129:414 25.
[137] Villacres Eriksson M, Bergstr m Mollaogl M, K berg H, Morein B. In ol e ment of IL2 and interferon gamma in the imm ne response ind ced b infl en a ir s iscoms. Scand J Imm nol 1992;36:421 6.
[138] Coffman RL, Lebman DA, Rothman P. Mechanism and reg lation of isot pe s itching. Ad Imm nol 1993;52:229 70.
[139] Behbo di S, Morein B, Villacres Eriksson M. *Quillaja* saponin form lations hat stim late proinflammat or c tokines elicit a potent acq ired cell mediated imm nit . Scand J Imm nol 1999;50:371 7.
[140] Sj lander A, Co JC, Barr IG. ISCOMs: an adj ant ith m ltiple f nctions. J Le koc Biol 1998;64(6):713 23.
[141] Rinaldo Jr CR, Torpe III DJ. Cell mediated imm nit and imm nos p pression in herpes simple ir s infection. Imm nodeficienc 1993;5(1): 33 90.
[142] Hein el FP, Rerko RM, Ahmed F, Pearlman E. Endogeno s IL 12 is req ired for control of Th2 c tokine responses capable of e acerbating leishmaniasis in normall resistant mice. J Imm nol 1995;155:730 9.
[143] Scott PA, Eaton WC, Ga se XD, Hondo ic k B. Earl IL 4 prod ction does not predict s sceptibilit to *Leishmania major*. E p Parasitol 1996;84: 178 87.
[144] Malo K, Donachie AM, Mo at AM. Ind ction of Th1 and Th2 CD4+ T cell responses b oral or parenteral imm ni ation ith ISCOMS. E r J Imm nol 1995;25:2835 41.
[145] Dotsika E, Karago ni E, S ndq ist B, Morein B, Morgan A, Villacres Eriksson M. Infl ence of *Quillaja saponaria* t riterpenoid content on the imm nomod lator capacit of Epstein Barr ir s Iscoms. Scand J Imm nol 1996;45: 261 8.
[146] Takahashi H, Takeshita T, Morein B, P tne S, Germain RN, Ber ofsk JA. Ind c tion of CD8+ c toto ic T cells b imm ni ation ith p rified HIV 1 en elope protein in ISCOMs. Nat re 1990;344(6269):873 5.
[147] Le TTT, Drane D, Malliaros J, Co JC, Rothel L, Pearse M, et al. C toto ic T cell pol epitope accines deli ered b ISCOMs. Vaccine 2001;19(32): 4669 75.
[148] W CA, Yang YW. Ind ction of cell death b saponin and antigen deli er . Pharm Res 2004;21(2):271 7.
[149] Van Binnendijk RS, an Baalen CA, Poelen MCM, de Vries P, Boes J, Cer ndolo V, et al. U tdeHaag, Measles ir s transmembrane f sion protein s nthesi ed de no o or presented in iscom is endogeno sl processed for HLA class 1 and class II restricted T cell recognition. J E p Med 1992;167:119 28.
[150] Sa ter B, Albert ML, Francisco L, Larsson M, Somersan S, Bhard aj N. Con seq ences of cell death: e pos re to necrotic t mor cells, b t not primar tiss e cells or apoptotic cells, ind ces the mat ration of imm nostim lator dendritic cells. J E p Med 2000;191(3):423 34.