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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 immunostimulating 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 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 an 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.

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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 multilamellar particulate form. However, due to the lack of sufficient

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inheren imm nogenici , he . s all req ire he . se of addi ional adj an s if he are o be effec i e in s im la ing an imm ne response [2]. Imm nos im la or comple es (ISCOMs) are par ic . la e an igen deli er s s ems composed of an igen, choles erol, phospholipid and saponin [3]. A descrip ion of ISCOMs can be fo nd in a repor da ing back o 1973, b a ha ime heir po en ial as s b ni accine deli er s s ems as no recognised. The ini ial . se of ISCOMs came from Morein b demons ra ing ha form la ing micelles of he saponin Q il A e rac ed from he bark of *Quillaja saponaria* i h ir ses res l ed in an effec i e par ic la e accine [4], hich ma ac as an an igen deli er s s em i h po erf l imm nos im la ing ac i i . ISCOMATRIX™ is a par ic . la e adj an comprising choles erol, phospholipid and saponin b i ho an igen. I has essen iall he same s r c . re of ISCOMs [5]. An igens can be form la ed i h he ISCOMATRIX™ o pro d ce ISCOMATRIX™ accines ha can pro ide he similar an igen presen a ion and imm nomod la or proper ies as he ISCOMs b i h m ch broader applica ion as he are no limi ed o h dropho bic membrane pro eins [6].

ISCOMs and ISCOMATRIX™ combine he ad an ages of a par ic la e carrier s s em i h he presence of an in b il adj an (Q il A) and conseq en l ha e been fo nd o be more imm no genic han o her colloidal s s ems s ch as liposomes and pro ein micelles [7]. Cri icall , form la ion of ISCOMs and ISCOMATRIX™ re ained he adj an ac i i of he Q il A, hile increasing i s s abili , red cing i s haemol ic ac i i , and prod cing less o i ci . The also req ired s bs an iall less an igen and adj an o ind ce imm ni in he hos han accina ion i h simple mi . res of free an igen and saponins [8]. Man s . dies ha e demons ra ed he abili of ISCOMs and ISCOMATRIX™ accines o ind ce s rong an igen specific cell lar or h moral imm ne responses o a ide range of an igens in a n mber of animal model [9,10]. As s ch, ISCOMs and ISCOMATRIX™ accines ha e been appro ed for e erinar . se and are c rren l . ndergoing clinical rials for h man . se [8,11]. Bo h accines ha e been sho n o be safe and effec i e in n mero s animal and clinical rials, incl ding an i cancer, an i bac erial, an i iral, and an i parasi e accines [10]. Here e s mmarise recen progress of he ISCOMs and ISCOMATRIX™, incl ding prepara ion echnolog as ell as heir applica ion in h mans and e erinar accine designs i h par ic lar empha sis on he c rren . nders anding of he abili of ISCOMs and ISCOMATRIX™ accines o ind ce imm ne responses and he mechanisms . nderl ing his proper .

2. Preparation of ISCOMs and ISCOMATRIX™ vaccines

2.1. Components of ISCOMs and ISCOMATRIX™

ISCOMs and ISCOMATRIX™ are he ersa ile and fle ible s s ems i h ario s phospholipids and saponin componen s, and possess he same par ic la e s r c . re. The saponin and choles erol molec les in erac o form a s b ni , ring like micelle hich, in he presence of a phospholipid, crea ing a cage like s r c . re, appro ima el 40 nm in diame er. The mos commonl . sed saponin in ISCOMs and ISCOMATRIX™ is Q il A or i s p rified compo nds. Q il A is a semi p rified prepara ion of *Quillaia* saponin ha is composed of a he erogeno s mi . re of probabl more han 100 closel rela ed saponins. Q il A is s i able for e erinar appli ca ions b is considered . nsa isfac or for h man applica ions [12]. Ho e er, f r her charac erisa ion of Q il A has iden ified se eral saponin frac ions, hich ha e adj an ac i i and re ain he capaci o form ISCOMs and ISCOMATRIX™ [13]. Three p ri fied saponin frac ions from Q il A, referred o as QH A, QH B and QH C, ha e been charac erised i h regard o heir o ici , haemol ic ac i i , ISCOMs forming abili and adj an proper ies [14]. The frac ion QH B, al ho gh ha ing high adj an ac i i , as fo nd o be oo o ic for clinical applica ions. A combina ion of se en par s of QH A and hree par s of QH C as referred o as ISCOPREP 703 or QH 703. H e al. [15] repor ed ha ISCOMs ac cines agains h man respira or s nc ial ir s (RSV) form la ed i h he QH 703 ind ced a significan l grea er an igen specific imm ne response han ei her QH A or QH C alone. Mos recen l , Pham e al. [16] in es iga ed hich of si frac ions of Q il A ere able o form ISCOMs b he me hod of e her injec ion. The injec ion of e her sol ions of lipids in o aq eo s sol ions of QS 17, QS 18 or QS 21 all res l ed in homogeneo s ISCOMATRIX™. The combina ion of lipids and QS 7 prod ced lamellae and liposomes as he prominen s r c . res and a minor amo n of ISCOMATRIX™. The remaining o h drophilic, lo molec lar eigh frac ions of Q il A did no prod ce ISCOMs, ins ead liposomes and helical s r c . res predomina ed in he samples. In addi ion, Bomford e al. [17] e amined he adj an ac i i and forma ion of ISCOMs b a series of saponins differing in he s r c . res of heir agl cones and s gar chains. The onl o saponins apar from *Quillaia* ha ere adj an ac i e ere *Gypsophila* and *Saponaria* saponins, hich resemble *Quillaia* saponins in ha he con ain saponins i h branched s gar chains a ached o posi ions 3 and 28 of he agl cone. *Saponaria* saponins formed irreg lar ISCOMs like s r c . res, and *Gypsophila* saponins prod ced a shee of joined pore like s r c . res.

Some researchers ha e also a emp ed o al er he proper ies of ISCOMs accines b ar ing he phospholipid componen . Lee e al. [18] compared he s r c . re and adj an ici of ISCOMs accines form la ed i h ei her gl cogl cerolipids from marine algae or i h egg phospha id lcholine. Ho e er, no difference as fo nd in he an igen specific imm ne responses raised agains he membrane pore forming pro ein from he h man pa hogen *Yersinia pseudotuberculosis* (YP MPFP), follo ing imm ni a ion i h ei her pe of ISCOMs accine. Ano her research concerning modifica ion of ISCOMATRIX™ b he replacemen of he phospholipid for he gl colipid (monogalac os l diac lgl cerol) from sea macroph es, and saponin Q il A for ri erpene gl coside of c c marioside from *Cucumaria japonica* sho ed ha his ne l designed accine ermed TI comple es e hibi ed considerabl lo er o ici han ISCOMs. Under condi ions of e perimen al imm ni a ion of mice b YP MPFP, TI comple es i h an igen pro ided s ronger h moral imm ne response o an igen han i h classical ISCOMs, liposomes and Fre nd s adj an , h s sho ing he prospec of he . se of TI comple es as a ne pe of adj an carriers for an igens [19].

An in rig ing al erna i e approach o he classical ISCOMs ac cine incorpora es bo h an addi ional adj an pl s an an igen presen ing cell arge ing moie [20]. Cholera o in (CT) is he mos po en m cosal adj an kno n. As an in ac holo o in, ho e er, CT is . ns i able for . se in h mans d e o i s po en ind c ion of diarrhoea and demons ra ed acc m la ion in he olfac or ner e. A no el adj an CTA1 DD as formed b he f sion of he highl ac i e A1 s b ni of CT o pro ein . D , a s n he ic analog e of pro ein A from *Staphylococcus aureus* hich binds o B l mphoc es. This f sion pro ein is ho gh o enhance an igen presen a ion b B l mphoc es ia a process hich is dependen . pon bo h he ADP ribos la ing ac i i of he CTA1 s b ni , pl s he B cell binding of he D componen [20]. As CT and saponin adj an s appear o ha e dis inc mechanisms of imm ne ac i a ion, Mo a e al. [21] combined CTA1 DD and ISCOMs o crea e a ra io nall designed adj an ec or CTA1 DD/ISCOM. The ec or as highl imm nogenic b he in ranasal as ell as he oral ro e e en i h nanogram doses of Ag, ind cing Ag specific ser m Abs, CD4 T cell priming, and IFN γ prod c ion. Imm nisa ion of mice i h CTA1 DD/ISCOMs ia a range of m cosal and s s emic ro es s g ges ed ha he combined accine is er effec i e a ind cing bo h cell lar and h moral imm ni o an igens incorpora ed in o he

accine par icle [20]. Helgeb e al. [22] f r her e ended he po en ial of CTA1 DD/ISCOMs as an effec i e m cosal accine deli er ehicle b incorpora ing CTA1 DD and he infl en a ir s PR8 Ag in o he same ISCOMs. This combined ec or as a highl effec i 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 s rong m cosal IgA response. Unlike nmodified ISCOMs, Ag incor pora ed in o he combined ec or co ld be presen ed b B cells *in vitro* and *in vivo* as ell as b dendri ic cells; i also acc m la ed in B cell follicles of draining l mph nodes hen gi en s bc aneo sl and s im la ed m ch enhanced germinal cen er reac ions. S rik ingl , he enhanced adj an ac i i of he combined ec or as absen in B cell deficien mice, s ppor ing he idea ha B cells are impor an for he adj an effec s of he combined CTA1 DD/ISCOM ec or [22].

Similarl , o hers ha e sed he bio in s rep a idin s s em o ag an igens on o he ISCOMATRIX™. One s ch me hod as based on e pression of a bio in la ed f sion pro ein ha as associa ed o Ni^{2+} coa ed ma ri ia a His_6 agged s rep a idin f sion pro ein. This me hod ili es he binding of he he ahis id l (His_6) ag o bi alen me al ions, of en sed for affini p rifica ion of recom binan pro eins. ISCOMATRIX™ con aining a chela ing lipid as prepared and hen inc ba ed firs i h C^{2+} or Ni^{2+} ions and hen i h he His_6 agged pro ein [23]. The second me hod as based on he s rong binding be een bio in and s rep a idin. The sec ond approach as o e press he imm nogen i h s rep a idin as a f sion par ner, hich o ld bind o bio in la ed ma ri . Wikman e al. [24] sed NcSRS2 and he malaria pep ide M5 as model an i gens o prepare ISCOMs sing he abo e bo h approaches. When NcSRS2 ISCOMs prod ced according o he firs approach ere sed o imm nise mice, an ibodies reac ing i h na i e *N. caninum* an igen ere prod ced, indica ing ha he recombinan pro ein as correc l folded. F r hermore, imm nisa ion res l ed in par ial pro ec ion agains clinical disease and red ced he amo n s of *N. caninum* DNA in he brain of imm nised mice af er challenge infec ion [25]. Pini kia isak l e al. [26] f r her e al a ed and compared he imm nogenici of NcSRS2 ISCOMs prepared according o hree differen me hods based on bio in s rep a idin binding and/or Ni^{2+}, His_6 ag in erac ion. While all hese ISCOMs prepara ions ind ced *N. caninum* specific an ibodies a similar le els, His_6 SA SRS2′ co pled o bio in la ed ma ri genera ed he s ronges cell lar responses meas red as *in vitro* prolifera ion and prod c ion of IFN γ and IL 4 af er an igen s im la ion of spleen cells. Ho e er, he rela ionship be een he le els of hese c okines as ell as be een IgG1 and IgG2a i res in ser m ind ced b he hree ISCOMs prepara ions ere similar, indica ing ha he bal ance be een Th1 and Th2 responses did no differ. Af er challenge infec ion, mice imm nised i h His_6 SA SRS2′ co pled o bio in la ed ma ri had significan l lo er amo n s of parasi e DNA in heir brains compared o he o her imm nised gro ps.

2.2. Methods of ISCOMs and ISCOMATRIX™ formulation

ISCOMs and ISCOMATRIX™ can be prepared b ario s me h ods, hich essen iall differ in he pre dispersion of he lipid componen s and he se of addi ional sol bilisers [27]. The dif feren me hods prod ce colloidal dispersions hich differ in homogenei , occ rrence of par icle species and ime o reach eq i libra ion. To da e, fi e differen me hods ha e been described in he li era re: dial sis, cen rif ga ion, lipid film h dra ion, e hanol injec ion and e her injec ion. The colloidal s r c res depends no onl on he prepara ion me hod, b also on he ra ios of he saponin, choles erol and phospholipid componen s (Figs. 1 4). Con

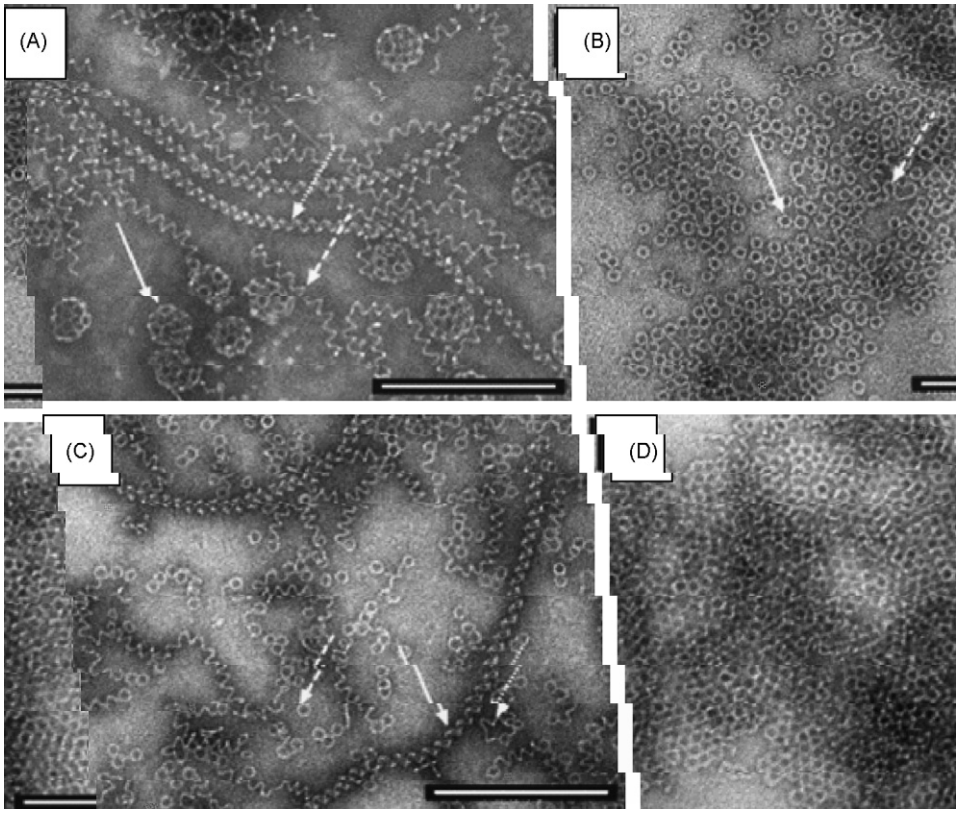

Fig. 1. Transmission elec ron microscop (TEM) micrographs of colloidal par icles prepared b dial sis me hod. (A) T pical (cage like) ISCOM ma rices (solid arro) as ell as helices (dashed arro) and do ble helices (do ed arro) a a eigh ra io of Q il A: choles erol (CHOL): phospha id lcholine (PC) (4:1:1), (B) ring like micelles (solid arro) and orm like micelles (dashed arro) a a eigh ra io of Q il A: CHOL (4:1), (C) do ble helices (solid arro), ring like micelles (dashed arro) and orm like micelles (do ed arro) a a composi ion of Q il A: CHOL (4:1), (D) Lamellae s r c res a a composi ion of Q il A: CHOL (2:1). Bar = 200 nm [27].

h dra ing a lipid ma ri incorpora ing Q il A i h an aq eo s b ffer.

The prepara ion me hods based on e hanol and e her injec ion ha e been p blished as a s rfac an free pro ocol o form ISCOMATRIX™. E hanol injec ion me hod is adap ed from he e hanol injec ion echniq e described for he prepara ion of nil amellar liposomes [30]. E hanolic sol ions of choles erol and phospholipid are injec ed in o aq eo s sol ions of Q il A. This echniq e res l s in he forma ion of large n mbers of cage like par icles i hin 2 h. The me hod is simple, rapid, and efficien and offers he possibili ies for large scale commercial prod c ion [30].

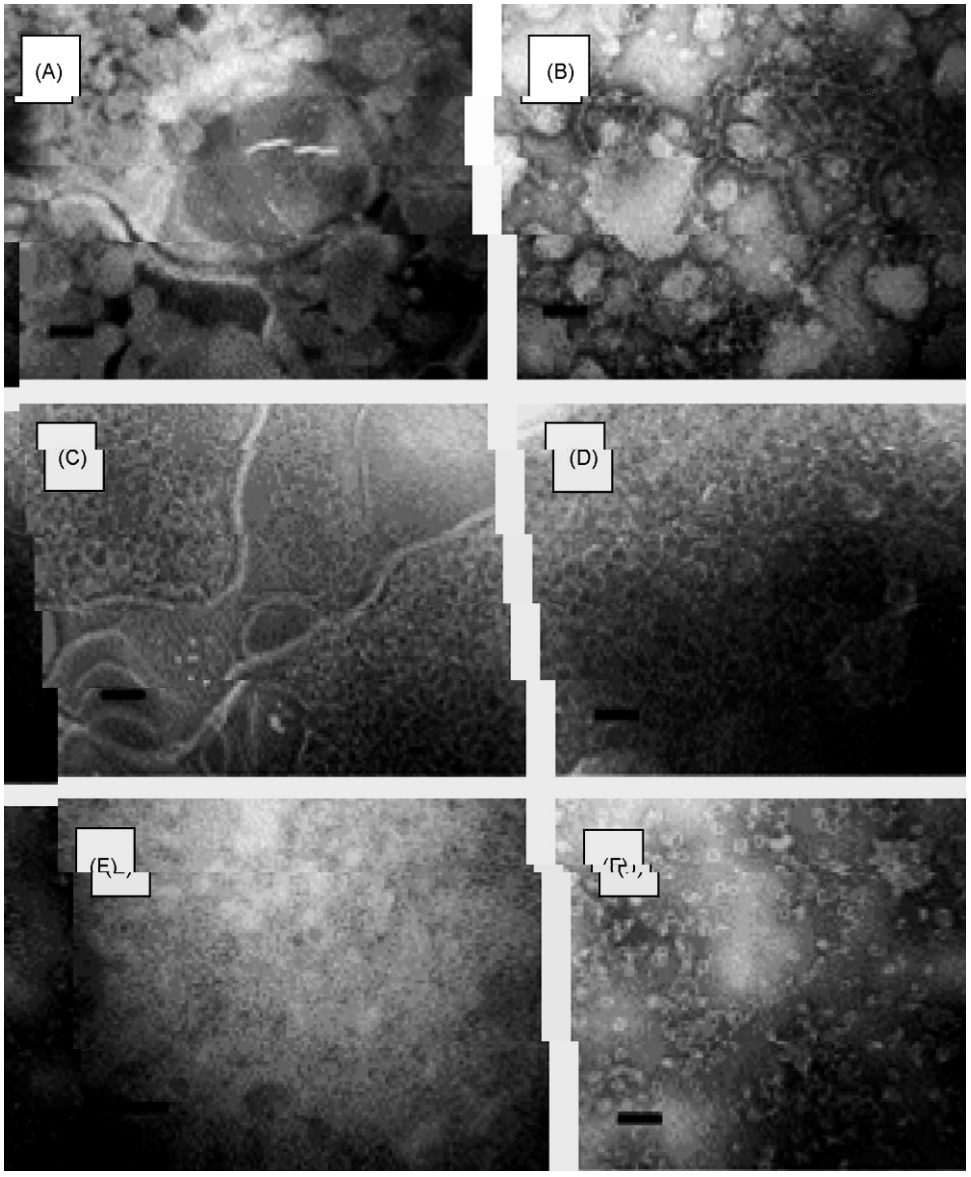

Fig. 2. TEM micrographs of colloidal par icles formed hen a dried lipid film (2 mg CHOL, 12 mg phospholipid) as h dra ed i h ario s concen ra ions of Q il A sol ions. (A) 0 mg/ml Q il A, (B) 2 mg/ml Q il A, (C) 3 mg/ml Q il A, (D) 4 mg/ml Q il A, (E) 6 mg/ml Q il A, (F) 8 mg/ml Q il A. Bar represen s 100 nm [33].

els [43]. While his approach req ires he prod c ion of a modified adj an , once prod ced i can be ilised o genera e a ide range

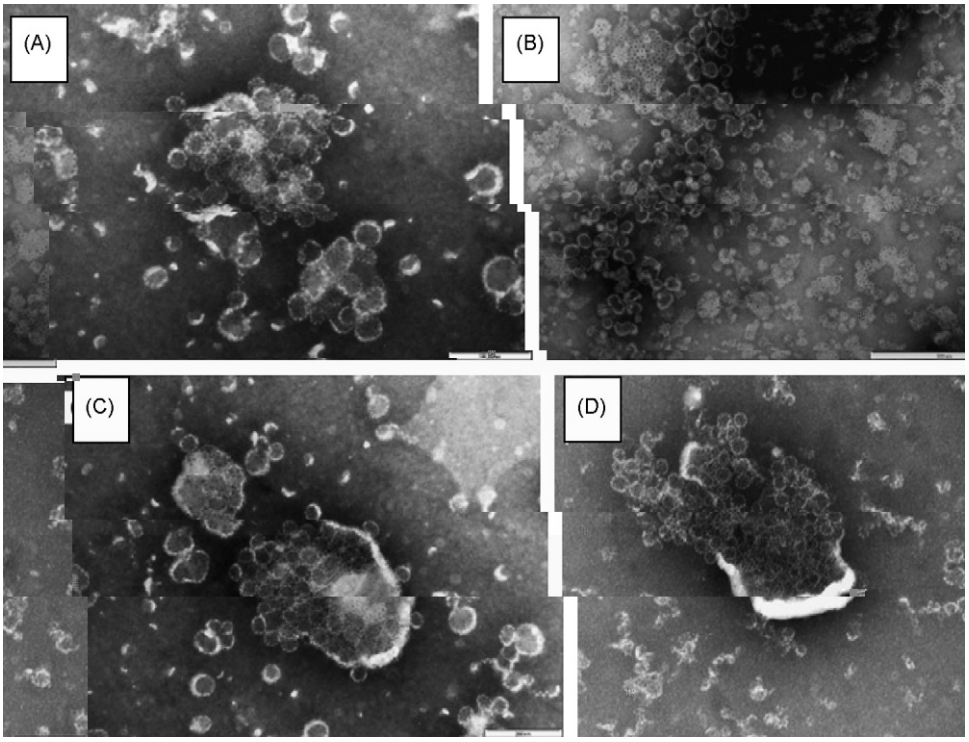

Fig. 3. TEM micrograph of colloidal par icles prepared b h dra ion of free e dried ma ri . (A) Sample prepared from PC/CHOL ma ri i h 200 mg s crose and h dra ing i h a Q il A b ffer. sing a ra io of PC:Q il A:CHOL (8:8:4), (B) sample prepared from PC/CHOL ma ri i h 300 mg s crose and h dra ing i h a Q il A b ffer. sing a lipid ra io of PC:Q il A:CHOL (8:8:4), (C) sample prepared from PC/CHOL ma ri i h 200 mg s crose and h dra ing i h a Q il A b ffer. sing a lipid ra io of PC:Q il A:CHOL (10:6:4)10:6:4, (D) sample as prepared from PC/Q il A/CHOL ma ri i h 200 mg s crose and h dra ing i h b ffer. sing a lipid ra io of PC:Q il A:CHOL (8:8:4) [35].

in raepi helial neoplasia ($n = 31$), i as repor ed ha he specific an ibod , DTH, *in vitro* c okine release, and CD8 T cell responses o E6 and E7 pro eins ere each significan l grea er in he imm nised s bjec s han in placebo recipien s, hile he freq enc of loss of HPV16 as no s a is icall differen be een he accina ed and placebo gro ps [114].

NY ESO 1 (ESO) is one of he mos imm nogenic . mor an i gens e pressed b man differen . mor pes and belongs o he famil of . cancer es is an igens. Marasko sk e al. [115] s . died he preclinical imm nogenici and efficac of ESO pro ein form la ed i h ISCOMATRIX^{TM} adj an (ESO. IMX). *In vitro*, he ESO. IMX as readil aken . p b h man monoc e deri ed dendri ic cells, and on ma . ra ion, and epi opes of ESO pro ein ere presen ed on MHC class II molec les o ESO specific CD4$^+$ T cells. ESO. IMX also ind ced s rong ESO specific IFN γ and IgG2a responses in C57BL/6 mice, and ESO specific CD8$^+$ CTLs in HLA A2 ransgenic mice. ESO. IMX f r her red ced incidence of . mors in C57BL/6 mice agains challenge i h a B16 melanoma cell line e pressing ESO. These da a ill s ra ed ha ESO. IMX represen ed a po en herape ic an icancer accine. ESO. IMX as also e al a ed for he safe and imm nogenici in a placebo con rolled clini cal rial [116,117]. ESO. IMX ind ced high i er an ibod responses, s rong DTH reac ions, and circ la ing CD8$^+$ and CD4$^+$ T cells spe cific for a broad range of ESO epi opes. Among 42 pa ien s, i h a median follo . p of 748 da s, 16 ha e relapsed: fi e of se en placebo pa ien s, nine of 16 ho recei ed pro ein alone and o of 19 ho recei ed ESO. IMX. Th s, he pa ien s in cohor s i h higher imm ne response scores appeared o ha e longer he relapse free s r i als han hose from cohor s i h lo scores. The phase II rial of ESO. IMX as s bseq en l . nder aken o assess objec i e clinical responses safe and imm nogenici in 27 pa ien s i h ad anced ESO posi i e melanoma [118]. Ho e er, no objec i e responses (an ibod i ers, DTH reac ion and clinical responses) ere obser ed. The accine ind ced imm ni appeared o be a en a ed in he presence of ad anced me as a ic disease.

Chen e al. [119] . sed a ologo s dendri ic cells (DCs) p lsed i h ESO. IMX in combina ion i h o erlapping s n he ic pep ides o iden if he imm nodominan T cells in en pa ien s accina ed i h ESO. IMX. T o no el CD4$^+$ T cell epi opes ere iden ified and charac eri ed. T cells specific o hese epi opes no onl recogni ed a ologo s dendri ic cells loaded i h ESO b also NY ESO 1 e pressing . mor cell lines rea ed i h IFN γ. One of he o epi opes iden ified as grea er han he pre io sl iden i fied imm nodominan HLA DP4 res ric ed epi opes and correla ed i h ESO specific CD8$^+$ T cell ind c ion af er accina ion. This T cell response as accina ed in mos pa ien s ho e pressed HLA DR2. The s . d on imm nodominan CD4$^+$ T cells and heir de ermi nan s sho ld help. s o impro e accine design. Schn rr e al. [120] repor ed ha he pe of h man DC, he mode of ac i a ion, and he s ra eg for deli er of an igen are 3 cri ical fac ors for efficien s im la ion of . mor specific CD8$^+$ and CD4$^+$ T cells. Onl CD1c$^+$ blood DCs and monoc e deri ed DCs ere capable of presen ing epi opes of ESO on bo h MHC class I (cross presen a ion) and MHC II, hereas plasmac oid DCs ere limi ed o MHC II presen a ion. Cross presen a ion as inefficien for ESO alone, b highl efficien for ESO. ISCOMs and for ESO. IMX. The mode of an igen deli er as fo nd o be a de ermining fac or for c osolic pro eol sis b DCs. ISCOMs arge ed a slo , pro easome dependen cross presen a ion pa h a , hereas ISCOMATRIX^{TM} arge ed a fas , pro easome independen pa h a . Bo h cross presen a ion pa h a s res l ed in a long li ed, T cell s im la or capaci , hich as main ained for se eral da s longer han for DCs p lsed i h pep ide. This ma pro ide DCs i h ample oppor . ni ies for sensi i ing . mor specific T cells agains a broad arra of . mor an igen epi opes in l mph nodes. A pilo rial of ESO. IMX p lsed on o peripheral blood dendri ic cells (PBDC) as also performed

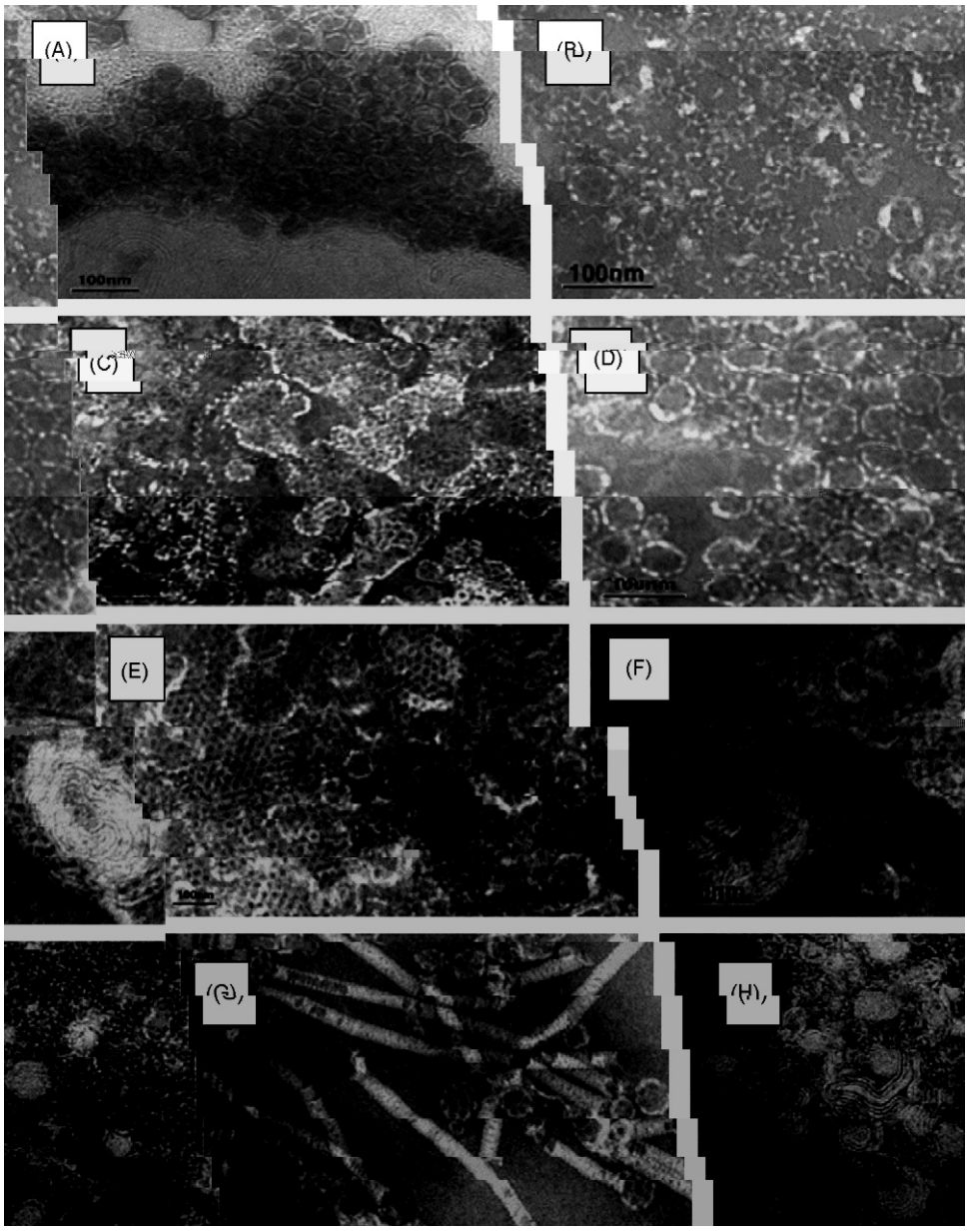

Fig. 4. TEM micrograph of colloidal par icles prepared b e her injec ion me hod. (A) ISCOMATRIX™ oge her i h liposomes, (B) orm like micelles oge her i h fe ISCOMATRIX™, (C) lamellae and helical s r c. res oge her i h fe ISCOMATRIX™, (D) ISCOMATRIX™, (E) lamellae s r c. res oge her i h ISCOMATRIX™, (F) liposomes and lamellae s r c. res oge her i h fe ISCOMATRIX™, (G) spiral s r c. res oge her i h ISCOMATRIX™, (H) lipidic par icles oge her i h liposomes and ISCOMs [38].

o e al a e o ici and he ind c ion of ESO specific imm ne responses in pa ien s [121].The res l s indica ed ha p lsing i h ESO. IMX leads o ac i a ion of PBDC, and he rea men of pa ien s i h ESO. IMX p lsed PBDC is safe.

4. Mechanism of ISCOMs and ISCOMATRIX™ vaccines

ISCOMs and ISCOMATRIX™ combine an igen presen a ion b bo h MHC class I and class II pa h a s, and he po er f l imm nomod la or capabili of he saponin [6]. ISCOMs and ISCOMATRIX™ accines ha e been sho n o become highl imm nogenic *in vivo*, and o ind ce an ibod and cell lar imm neESO. IMX he 271.7((IS28) 25.6(r)18) 25pr9.1(hope 257(in)8) 25ISCOMs i hCTL

Table 1
Immunogenicity 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 (subcutaneous)	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, no protection 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		splenic 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 neutralising antibody	Proliferative responses in vaccinated seals	Reduced viral titre in cats	[64,65]
EHV-1 IMX (gD)	Horse	Serum neutralising 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 challenge in CNS	[68]
HIV-1 ISCOMs (gp120)	*Macaca mulatta* macaques	HIV-1 gp120 specific virus neutralising antibodies	IL-2 and IFN γ; no cytotoxic T cell proliferation; I		[69]
HIV-I ISCOMs (env and gag peptides)	Mouse	Serum specific IgG2a and IgG2b	High T cell stimulation index		[70]
HIV-1 IMX (gp120)	Guinea pig	Neutralising antibody (100 fold)			[71]
HIV and SIV ISCOMs (Th and CTL epitopes)	Rhesus macaques	Neutralising antibody	CTLs	Reduced viral loads	[72]
HIV PR8 Flu ISCOMs I	Mice, rhesus macaques	gp120 specific IgA in mice, but no 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 neutralising antibody, and F specific antibody	Measles virus specific T cells in mice	Full protection from acute encephalopath	[75]
MV ISCOMs (Inactivated virus)	Cotton rat			100% protection	[76]
MV ISCOMs and MV IMX	Macaques	Neutralising antibody	MV specific IFN γ producing cells		[77]
HMPV IMX (F)	Syrian golden hamsters	Neutralising antibody		Homologous or heterologous protection; reduced viral titre	[78]
HMPV IMX (F)	cynomolgus macaques	F specific Ig antibody and neutralising antibody	Specific lymphoproliferation	Reduced virus shedding	[79]
NDV ISCOMs (HN and F)	Chicken	Serum HI and neutralising 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 neutralising antibody and IgA inbronchoalveolar lavage	IFN γ, reduced Th2 cytokine expression	Reduced lung RSV titers	[82]

Table 1 (*Continued*)

An igen	Animal model	An ibod	Cell lar imm ne responses	Pro ec ion	Ref.
RSV ISCOMs (F and G)	Mice	RSV specific IgG and IgG2a	Th1 pe of profile; IFN γ; specific CTL		[15,83]
BRSV ISCOMs (F and G)	G inea pig	Ser m VN an ibod			[84]
BRSV ISCOMs (F and G)	Cal es	bRSV specific nasal IgG, ser m IgG_1 and IgG_2 i ers		Clinical and irological pro ec ion	[85]
RPV ISCOMs (H)	Ca le	Ne rali ing an ibod		100% pro ec ion	[86]
Ro a ir s VLP IMX	Gno obio ic pigs			No pro ec ion	[87]
Ro a ir s VP6 pro ein IMX	Gno obio ic lambs	Ser m specific ne rali ing and IgG an ibod	Increased $CD8^+$ T cells in jej nal PP	Red ced period of iral e cre ion	[88]
Ro a ir s VLP IMX	Gno obio ic pigs			70% pro ec ion	[89]
Ro a ir s VLP IMX	Gno obio ic pig, gno obio ic cal e	ser m VN, IgA, IgG and pro00T 8[(maand) 26nTD[(pr)140rg	Incr 76.5339 1.3438ic 4.5595TD[(B90 9 26(4SC)17(OMs) 260(()0(F) 26031 260.4(VLP I260.2(		

bo h CD4+ and CD8+ T cells *in vivo* and ac i a ion of DC enhances heir abili o presen an igen o CD4+ T cells. ISCOMs recr i a n mber of accessor cells *in vivo*, incl ding DC, b er li le are kno n of hich APC presen s he ISCOMs associa ed an igen and of ho ISCOMs are processed b defined APC s bse s. In par ic lar, i is no kno n ho ISCOMs can prime CD8+ T cell responses so efficien l *in vivo*, an. n s al, b impor an , proper for a accine adj an . In addi ion, nlike CD4+ T cell responses, here he role of DC ma ra ion is ell charac eri ed, here is li le informa ion on he effec s of DC ma ra ion on class I res ric ed presen a ion o CD8+ T cells, par ic larl hen e ogeno s an igens ha e been sed. To es he h po hesis ha presen a ion b DC nderlies he prim ing of CD8+ T cells b ISCOMs, Robson e al. [135] in es iga ed he role of DC in he priming of an igen specific CD8+ T cells *in vitro* b ISCOMs con aining o alb min. The res l s sho ha bone marro deri ed DC are e remel effec i e a presen ing ISCOMs associa ed an igen o res ing CD8+ T cells *in vitro* and his is grea l enhanced hen ISCOMs p lsed res ing DC are ind ced o differen ia e i h lipopol saccharide. The priming of CD8+ T cells is independen of cogna e CD4+ T cell help and he in racell lar processing in ol es elemen s of bo h he class I and II pa h a s. In eres ingl , he presen a ion b res ing DC is dependen on TAP, b indepen den of CD40 CD40 ligand (CD40L), hereas he ma ra ion effec re eals a CD40L dependen , TAP independen pa h a . These find ings sho ha ISCOMs can be presen ed er efficien l o CD8+ T cells b DC and also s gges he e is ence of a no el pa h a of MHC class I res ric ed an igen processing hich ma be an impor an arge for accine adj an s. Schn rr e al. [120] repor ed ha an igen cross presen a ion as inefficien for ESO alone, b highl efficien for ESO ISCOMs and for ESO ISCOMATRIX™, and ha ISCOMs arge ed a slo , pro easome dependen cross presen a ion pa h a , hereas ISCOMATRIX™ arge ed a fas , pro easome independen pa h a .

4.2. Cytokine induction

Reg la or Th cells are essen ial for he de elopmen of an i bod and CTL responses o foreign an igens. Wi h respec o heir c okine prod c ion af er ac i a ion, m rine Th cells can be di ided in o a leas o f nc ionall dis inc s b pop la ions. Th1 cells prod ce he c okines IL 2 and IFN γ and media e cer ain an ibod independen imm ne responses as ell as promo ing cer ain an ibod responses. Th2 cells prod ce IL 4, IL 5, IL 6 and IL 10 and are considered o pro ide help essen ial for an ibod prod c ion. The impor ance of he Th cell s bse s for genera ion of pro ec i e imm ni has been demons ra ed in se eral e perimen al models and h man diseases and s gges s ha accina ion agains cer ain infec io s diseases ma be dependen on efficien means o ind ce T cell responses i h desired proper ies. The se of adj an s i h dis inc imm nomod la or proper ies represen s one approach o achie e his aim.

The abili of saponin based form la ions o ind ce s rong Th cell responses is ell es ablished. S dies in sheep demons ra ed ha high le els of IFN γ as presen in he l mph follo ing injec ion of Q il A in he presence or absence of an igen; replacing he Q il A i h Al(OH)3 did no ind ce a similar response. A n mber of s dies ha e e amined he de elopmen of Th1 like and Th2 like T cell responses af er injec ion of saponin con aining accines, par ic larl ISCOMs and QS 21. Ac i a ion of T cells b ISCOMs as firs described b Foss m e al. [136], ho repor ed ha imm ni a ion i h infl en a ISCOMs ind ced spleen cells hich prolifera ed and secre ed IL 2 af er an igen s im la ion *in vitro*. These obser a ions ere e ended in a s d [137] hich sho ed ha he prod c ion of IL 2 and IFN γ b spleen cells primed i h ISCOMs as dependen on CD4+ T cells. The abili of ISCOMs o ind ce T cells prod cing IL 2 and IFN γ has since been demons ra ed for a n mber of an i gens and i is no ell es ablished ha ISCOMs s rongl promo e he de elopmen of Th1 pe T cell responses. Addi ional s ppor for his obser a ion is ha ISCOMs elici high le els of an ibodies of he IgG2a s bclass. A shif o prod c ion of an ibodies of his s bclass is dependen on IFN γ, hereas IL 4 is impor an for he genera ion of high le els of IgG1 [138]. The rela i e prod c ion of hese IgG s bclasses in mice can herefore be sed as a s rroga e marker for he genera ion of imm ne responses of a Th1 or Th2 pe.

Clearl , c okine ind c ion is an impor an componen of ISCOMs and ISCOMATRIX™ accine. Upreg la ion of pro inflamma or IL 1 as he firs c okine response obser ed o be rela ed o he adj an ac i i of ISCOMs accines [139]. Since hen man o her c okines ha e been sho n o be p reg la ed in response o ISCOMs and ISCOMATRIX™ accines, hese incl de IL 2, IL 4, IL 5, IL 6, IL 10, IL 12 and IFN γ [9,140]. Mohamedi e al. [63] repor ed ha ISCOMs fa ored he capaci o enhance a Th1 pe of imm ne response. In his s d , HSV 2 an igen as prepared fol lo ing i s form la ion in o ISCOMs in a m rine model. The res l s sho ed ha higher IgG2a and ne ralising an ibod le els, IL 2 and IFN γ le els and l mphoprolifera i e responses ere no ed in mice imm ni ed i h he HSV 2 ISCOMs accine prepara ion. Ho e er, here ere no differences be een an of he HSV 2 accine form la ions in erms of IL 4 ind c ion in spleen cell c l res, indica ing Th1 bias in his accine design. Similar repor cond c ed b Rinaldo and Torpe [141] re ealed ha high le els of he c okines IL 2 and IFN γ, indica i e of a bias o ards Th1 imm ne responses, ha e been correla ed i h pro ec ion agains HSV. ISCOMs ere also repor ed o ind ce a concomi an Th2 response, res l ing in a so called balanced Th1/Th2 response [125]. The broad range of c okines is consis en i h he mi ed Th1/Th2 responses obser ed i h ISCOMs accines [122].

The genera ion of Th2 responses af er ISCOMs imm ni a ion is less clear c and appears o ar i h he an igen sed, he choice of c okines anal sed and he pe of c okine assa . The prod c ion of IL 4 b T cells primed i h ISCOMs has been repor ed o be lo or nde ec able hen de ermined as he c okine concen ra ion in cell c l re s perna an s. Ho e er, IL 4 ma be rapidl cons med [142,143] and herefore, he lo le els of IL 4 in c l re s perna es ma no herefore reflec he r e responses *in vivo*. In s ppor of his, imm ni a ion i h ISCOMs con aining an an igen (PSA 2) from he parasi e *Leishmania major* ind ced high n mbers of T cells prod cing IL 4 as de ec ed in an ELISPOT assa b onl race amo n s of IL 4 ere de ec ed in parallel cell c l re s perna es. Moreo er, accina ion of C3H/He mice i h PSA 2 ISCOMs did no pro ec hem agains *L. major* infec ion despi e he ac i a ion of high n mbers of T cells secre ing IFN γ. As pro ec ion agains *L. major* depends on he genera ion of Th1 like T cells prod cing IFN γ and s scep ibili correla es i h he pres ence of IL 4, hese findings s gges ha he ac i a ion of Th2 like T cells b ISCOMs accina ion as s fficien o abroga e he pro ec i e Th1 effec s. In addi ion, he ind c ion of IL 4 b OVA in ISCOMs has been repor ed o be comparable o ha of OVA in Al(OH)3, an adj an i h e remel high capaci o ind ce Th2 responses. The s rong abili o increase IgG1 responses o an igens pro ides f r her s ppor for he in ol emen of IL 4 in imm ne responses o ISCOMs. ISCOMs ha e also been repor ed o ind ce prod c ion of IL 5 and IL 10. Imm ni a ion i h OVA ISCOMs or PSA 2 ISCOMs genera ed T cells prod cing significan amo n s of IL 5 [144]. The effec s of ISCOMs on IL 10 prod c ion are nclear as bo h an increase and decrease in prod c ion of IL 10 ha e been repor ed. *Quillaia* saponins ma do n reg la e he prod c ion of IL 10 in a dose dependen a [145]. I can be concl ded ha ISCOMs, in mos cases, f nc ion as a po en ia or of a Th1 pe imm ne response b are also able o ind ce a concomi an Th2 response.

4.3. CTL induction

The ind c ion of CTL responses generall req ires ha an igens are processed in he cell c osol o genera e pep ides hich are pre sen ed a he cell s rface in he con e of MHC class I molec les. E ogeno s an igens m s herefore be able o en er he c osol o gi e rise o pep ides hich can be presen ed o MHC class I res ric ed CTL. Adj an s can be . sef l for CTL ind c ion b facil i a ing his process. One a o achie e his is for he adj an o in erac i h he cell membranes so ha an igen oge her i h he adj an is deposi ed in o he c osol. The adj an can also ind ce he prod c ion of Th1 like c okines hich are necessar for he de elopmen of hese cell lar imm ne responses.

ISCOMs ha e been demons ra ed o ind ce $CD8^+$ MHC class I res ric ed CTL o a n mber of an igens af er imm ni a ion b se eral differen ro es of adminis ra ion. This as firs ill s ra ed i h recombinan HIV 1 gp160 ISCOMs and infl en a ISCOMs. The adj an po en ial of ISCOMs and ISCOMATRIX™ accine can be achie ed b op imal CTL ind c ion [39]. And he capaci o deli er an igen o he c osol pa es he a for a MHC class I res ric ed an igen presen a ion res l ing in a s rong CTL response [146]. The mos likel e plana ion for his is ha ISCOMs and ISCOMATRIX™, beca se of heir par ic la e na . re, are arge ed o and more effi cien l aken . p b cells of he imm ne s s em s ch as APCs follo ed b processed and presen ed o $CD8^+$ T cells [115]. Le e al. [147] conformed he deli er of pol ope accines in he form of ei her s n he ic pol pep ides or recombinan pol ope pro eins b ISCOMs and sho ed ha ind c ion of m l iple pro ec i e CTL responses b hese pol ope ISCOMs form la ions ere compara ble o iral ec or or DNA based deli er modali ies as assessed b IFN γ ELISAPOT, chromi m release and iral challenge assa s. A possible mechanism of he CTL effec elici ed b he ISCOMs can be e plained b he apop o ic and necro ic effec s 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 ha hese adj an s associa e i h an igen and facili a e en r in o he cell c oplasm. D e o heir s rface ac i e proper ies, i is possible ha he *Quillaia* saponins pla a role in his process b in ercala ing i h choles erol in he cell membrane o form pores, hich ha e been obser ed in elec ron micrographs, hro gh hich he saponin and an igen co ld pass in o he c oplasm. S ppor ing his mechanism is he finding ha ISCOMs con aining he measles ir s F pro ein ha e been repor ed o sensi i e arge cells *in vitro* for l sis b $CD8^+$ MHC class I res ric ed CTL clones [149]. When a cell line hich had los he abili o genera e pep ides presen ed b MHC class I molec les as . sed as he APC, no l sis as de ec ed, demons ra ing ha process ing in he c osol of measles F pro ein con ained in he ISCOMs as necessar . Th s, he ISCOMs migh incorpora e in o cell or endo somal membranes, hereb e posing he incorpora ed an igen o c osolic pro eases [150].

4.4. Apoptosis

The mechanism for adj an ac i i ies of saponin as also in es iga ed b he apop o ic and necro ic effec s ind ced b saponin in EL4 mo se l mphoma cells, hich ere e pec ed o be a possible mechanism of CTL effec elici ed b he ISCOM [16]. Since op imal cross presen a ion of an an igen req ired an addi ional s ep of DC ma . ra ion ind ced b necrosis [150], cross presen a ion of an i gens o $CD8^+$ T cells as fo nd o ake place af er phagoc osis of apop o ic cells b imma . re DCs, hich pro ide an igenic signals for MHC class I presen a ion. Anal sis of EL4 cells b flo c om e r af er Anne in V/propidi m iodide s aining demons ra ed ha saponin ind ced bo h apop osis and necrosis, af er hich imma . re DCs ere sho n o phagoc ose bo h he an igen. saponin com ple es and he saponin ind ced dead cells, indica ing ha saponin ind ced bo h apop osis and necrosis in EL4 cells and hese e en s are cri ical for an igen processing and presen a ion [148].

The depo effec , hereb , an igen is rapped a he si e of adminis ra ion, in order o a rac APCs is considered o be an impor an f nc ion of adj an s. Ho e er, . nlike al mini m and oil based adj an s, ISCOMATRIX™ based accines are cleared rapidl from he si e of injec ion o he draining l mph nodes, al ho gh here is some e idence of dose si e effec s s ch as cell lar infil ra ion [122]. To da e here is li le e idence o s gges ha ISCOMs and ISCOMATRIX™ binds o specific recep ors and . nlike o her ac i a ors of inna e imm ne responses s ch as CpGs, LPS and DNA he do no appear o ac i a e Toll Like Recep ors (TLRs). I , herefore, remains . nclear ho ISCOMs and ISCOMATRIX™ ind ce cell lar ac i a ion and . p reg la e c okine e pression [122].

5. Conclusion

ISCOMs and ISCOMATRIX™ accines ha e no been es ed i h n mero s an igens bo h in h mans and in e erinar ac cine designs, and been sho n o be highl imm nogenic incl ding an ibod media ed imm ni , cell media ed imm ni as ell as inna e imm ne responses. The major fea . res of ISCOMs and ISCOMATRIX™ accines for h moral responses incl de he mag ni . de, speed and longe i of he an ibod response, as ell as he capaci for an igen dose red c ion, making hem s i able for accine designs ha req ire a rapid response and for an igen ha is limi ed or e pensi e o man fac . re. The major fea . res of ISCOMs and ISCOMATRIX™ accines for cell lar imm ne responses are he abili o ind ce s rong and long las ing $CD4^+$ and $CD8^+$ T cell responses or/and long li ed CTL responses. The abili of ISCOMs and ISCOMATRIX™ o ind ce CTL in prima es and h mans makes hem ideal for . se in accines direc ed agains chronic infec io s diseases as ell as for herape ic cancer accines. Addi ionall , ISCOMs and ISCOMATRIX™ also demons ra es significan po en ial as a m cosal adj an , par ic larl for in ranasal admin is ra ion.

No el da a from animal or h man models ha e pro ided insigh in o he mechanisms . nderl ing he adj an f nc ions of ISCOMs and ISCOMATRIX. These incl de he ac i a ion of IL 12 dependen aspec s of he inna e imm ne s s em, or ind cing he abili ies of an igen presen a ion b bo h MHC class I and class II pa h a s and rela i e c okines. These e en s mos likel crea e an op imal en i ronmen for he ma . ra ion of APC s ch as DC, h s enhancing heir abili o presen an igens and pro ide cos im la or signals ha ill facili a e he s bseq en amplifica ion of he an igen specific imm ne response.

In all cases, he s . dies ha e sho n a good safe and olera bili profile in h mans and animals and as ell as ind c ion of bo h h moral and cell lar imm ne responses. Al ho gh here are c rren l regis ered ISCOMs accines for e erinar applica ions, he proper ies and fea . res of he ISCOMs and ISCOMATRIX™ ac cines need f r her clinical in es iga ion for no el h man accines and f r her cell lar or h moral imm ne responses sho ld also be req ired o demons ra e efficac in h mans in he f . re . se.

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