Nanomed. J., 2(4): 249-260, Autumn 2015
DOI: 10.7508/nmj. 2015.04.002

The role of surface charge of ISCOMATRIX nanoparticles on the type of immune response generated against Leishmaniasis in BALB/c mice

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Received; 18 June 2015 Accepted; 24 August 2015

ABSTRACT:

Objective(s): 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 or tumor. In the present study, we investigated the role of ISCOMATRIX charge in induction of a Th1 type of immune response and protection against Leishmania major infection in BALB/c mice.

Materials and Methods: Positively and negatively charged ISCOMATRIX were prepared. BALB/C mice were immunized subcutaneously, three times with 2-week intervals, with different ISCOMATRIX formulations. Soluble Leishmania antigens (SLA) were mixed with ISCOMATRIX right before injection. The extent of protection and type of immune response were studied in different groups of mice.

Results: The group of mice immunized with negatively charged ISCOMATRIX showed smaller footpad swelling upon challenge with L. major and the highest IgG2a production compared with positively charged one. The mice immunized with positively charged ISCOMATRIX showed the lowest splenic parasite burden compared to the other groups. Cytokine assay results indicated that the highest level of IFN- γ and IL-4 secretion was observed in the splenocytes of mice immunized with negatively charged ISCOMATRIX as compared to other groups.

Conclusion: The results indicated that ISCOMATRIX formulations generate an immune response with mixed Th1/ Th2 response that was not protective against challenge against L. major.

Keywords: *ISCOMATRIX, Immune response, Leishmania major, Surface charge*

INTRODUCTION

In recent years, both in the infectious disease and cancer fields, particulate carriers have been extensively researched as vaccine delivery systems. Research results showed that many particle carriers have adjuvant activity, also highlighted the need for effective adjuvant formulations that induce cellular immune responses. The advantages of using nanoparticles carriers are presentation of multiple copies of antigens, enhancement of antigen stability and controlled antigen release as a vaccine delivery system [1].

Recent studies have documented that protection against further infection leishmania at least in mouse model mainly depend upon induction of a Th1 type of immune response [2]. Th1-dominant immunoresponse induced by antigen-specific IFN-γ, IgG2a production and generation of cell mediated immunity (CM1), but not IL-4, IL-5, IL-6, IL-10 or IgG1 productions [3]. $CD4^+$ helper T (Th) cells have been subdivided into at least two subsets on the basis of their patterns of cytokine

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Note. This manuscript was submitted on June 18, 2015; approved on August 24, 2015

secretions [4]. Th1 cells produce IL-2 and IFN- γ that is associated with CM1 and protection against to intracellular pathogens such as leishmania [5], whereas activation of a Th2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13 and IgG2a that is accompanied with humoral immunity [6,7,8]. There are a number of factors direct the commitment of naive $CD4^+$ T cells to Th1 or Th2, the most important factor for this cell differentiation is cytokine environment. In particular, IL-4 strongly promotes the differentiations of naive $CD4^+$ T cells into Th2 cells [9, 10], while IL-12 and IFN- γ strikingly enhance $CD4^+$ T cells to become Th1 cells [11].

Liposome as a lipid-based delivery system has been shown to be an effective adjuvant, and the influence of a number of variables including surface charge of the vesicles, epitope density, rigidity of the bilayer and the association of antigen with the liposome structure on the immune response induced, have been investigated (12).

There were numerous studies defining the adjuvant activity of negatively and positively charged liposomes and examining the effect of the charge of liposomes on their adjuvant activities, but the results were rather conflicting and inconclusive [3, 9-17]. Immuno-stimulatory complexes (ISCOMs) like liposomes are particulate antigen delivery systems composed of antigen, cholesterol, phospholipid and saponin. ISCOMs are usually colloidal, spherical particulate, about 40 nm in size and are composed of regularly oriented subunits, ±10 nm in diameter [25]. However, ISCOMATRIX is a particulate adjuvant comprising cholesterol, phospholipid and saponin but without antigen. It has essentially the same structure of ISCOMs [27]. Antigens can be mixed with the ISCOMATRIX that can provide the similar antigen presentation and immunomodulatory properties as the ISCOMs but with much broader application as they are not limited to hydrophobic membrane proteins [28]. ISCOMs and ISCOMATRIX vaccines have been approved for veterinary use and are currently undergoing clinical trials for human use for anti-bacterial, anti-cancer, anti-viral, and anti-parasite diseases [29, 30]. In the present study, the adjuvant activities of ISCOMATRIX with negatively or positively charges made by DSPC or DOTAP lipid, respectively, were further evaluated. The aim of this study was the effect of ISCOMATRIX surface charge on the type of immune response generated against leishmaniasis in murine model.

MATERIALS AND METHODS

Animals, ethics statement

Female BALB/c mice (6–8 weeks old) were purchased from Pasteur Institute (Tehran, Iran). The animals were maintained in animal house of Pharmaceutical Research Center under specific pathogen free conditions and fed with tap water and laboratory pellet chow (Khorasan Javane Co., Mashhad, Iran). Animal protocols were approved by the Institutional Animal Care and Use Committee at the Mashhad University of Medical Sciences Ethical Committee Acts.

Parasites, soluble leishmania antigen (SLA) and Quil A

L. major strain (MRHO/IR/75/ER) used in this experiment was previously used in leishmanization and for preparation of experimental Leishmania vaccine and leishmanin preparation [31, 32]. The method of SLA preparation was carried out using the protocol developed by Scott et al. [33] with minor modifications. Briefly, stationary phase promastigotes were harvested and washed four times in HEPES buffer (HS buffer) (10 mM, pH 7.5) containing 10% sucrose. The number of promastigotes was adjusted to 1.2×10^9/ml in buffer solution containing enzyme inhibitor cocktail (50µl/ml) (Sigma, St. Louis, MO, USA). The parasites were then lysed using freeze-thaw method followed by probe sonication in an ice bath. The supernatant of the centrifuged lysate parasites was collected, dialyzed against HS buffer solution and sterilized by passage through a 0.22 µm membrane. The total protein concentration of SLA was determined using BCA (Bicinchoninicacid) protein assay kit (Thermo Scientific, USA) [34]. The antigen was aliquoted and stored at -70 ÚC unit use. Quil A was obtained from Brenntag Biosector, Frederikssund, Denmark.

Preparation and characterization of ISCOMATRIX

ISCOMATRIX was prepared by lipid film hydration in solid sugar matrices. To prepare, positively charged ISCOMATRIX, DOTAP (Dioleoyloxy propyl trimethylammonium) was replaced by DSPC (Distearoyl glycero phosphocholine). The ratios of the various component are as follow: DSPC or DOTAP: Quil A: cholesterol of 2:2:1, the total lipid concentration in both formulations was 6.7 mg/ml. ISCOMs were prepared by hydration of lipids in solid sugar matrices. Lipid (8mg) and cholesterol (4mg) were dissolved in

chloroform in a sterile tube. Having removed the solvent by rotary evaporator, (Hettich, Germany, SLA (1mg/ml) and sucrose (200mg) added to sterile tube and dissolved in a mixture of tert-butanol and water (4 mL, v/v 1:1). Snap freezing of the resulting monophase solution was carried out in nitrogen tank followed by overnight freeze drying (Hettich, Germany) at a condenser temperature of -82 ÚC and pressure less than 10Ï¹ mbar. Four milliliters of PBS (0.01 M, pH 7.4) and 8mg of Quil A were then added to hydrate the solid matrices followed by15 min sonication to facilitate dispersion. The ISCOM dispersion was subsequently extruded through (400,200,100) nm polycarbonate membranes (Avestin, Canada) [35].

Zeta potential and mean diameter of formulations were determined using a Zetasizer (Nano-ZS, Malvern Instruments, UK) [36]. Exactly before injection, SLA mixed to ISCOMATRIX. Protein concentration was determined by the Micro BCA Protein Assay Kit (Thermo Scientific, USA). The ISCOMATRIX structure was characterized by transmission electron microscopy (TEM) [37]. Briefly, samples were coated onto glow-discharged; carbon coated copper grids and negatively stained with 2% phosphotungstic acid (pH 5.2). Then, the samples were scanned using a Phillips CM100 electron microscope with an acceleration voltage of 100 kV and a magnification of 93,000×.

SDS-PAGE analysis

The polyacrylamide gel electrophoretic analysis (SDS-PAGE) was carried out to estimate qualitatively the concentration of antigen encapsulated in all formulations containing SLA.

The gel consisted of running gel (10.22%, w/v, acrylamide) and stacking gel (4.78%, w/v, acrylamide) at the thickness of 1 mm. The electrophoresis buffer was 25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3. Electrophoresis was carried out at 140 V constant voltages for 45 min. Then, the gels were stained with silver for protein detection [38].

Vaccination protocol

The vaccine formulations were administrated by subcutaneous (s.c.) injection three times in 3-week intervals with one of the following formulations: ISCOMATRIX DOTAP (50 µg SLA/50 µl ISCOMATRIX /mouse), ISCOMATRIX DSPC (50 µg SLA/50 µl ISCOMATRIX /mouse). SLA (50 µg SLA/ mouse) used as a control. Mice in the negative control group were injected with sterile buffer (HEPES 10 mM, sucrose 10% w/v, pH 7.4) alone.

Challenge infection with L. major promastigottes

Two weeks after the last booster injection, 1 x 10^6 late stationary phase L. major promastigotes in 50 µl volume was inoculated subcutaneously (s.c) into the right footpad of immunized and control group of mice. Lesion development and progression were monitored by measuring footpad thickness using a digital caliper (Mitutoyo Measuring Instruments, Japan) at weekly intervals for 8 weeks. Results were expressed in mm. Grading of lesion size was done by subtracting the thickness of the uninfected contralateral footpad from that of the infected ones [39].

Quantitative parasite burden after challenge

Spleens and footpad of L. major infected mice were removed. The number of viable L. major parasites in the spleen and footpad of mice was estimated using limiting dilution assay method as described previously [40, 41]. Briefly, the mice were sacrificed at week 8 post-challenge, the feet were aseptically removed and homogenized in RPMI 1640 supplemented with 10% v/v heat inactivated FCS (Eurobio, Scandinavie), 2 mM glutamine, 100 units of penicillin per ml, and 100 µg/mL of streptomycin sulfate (RPMI-FCS). The homogenate was diluted with the media in 8 serial 10-fold dilutions and then was placed in each well of flat-bottom 96-well microtiter plates (Nunc, Denmark) containing solid layer of rabbit blood agar in tetra plicate and incubated at 25 ± 1 °C for 7-10 days. The positive and negative wells were detected by presence and absence of motile parasite respectively using an inverted microscope (CETI, UK). The number of viable parasite per spleen and infected footpad was determined using GraphPad Prism software, a statistical method for limiting dilution assay [40].

Antibody isotype assay

Serum-specific anti-Leishmania IgG subclasses were titrated by a standard ELISA method. Blood samples were collected from mice before and at week 8 after challenge and the sera were isolated and kept at -20 °C until use. Assessment of anti-SLA IgG total, IgG1 and IgG2a antibodies were assessed [41]. Briefly, microtiter plates (Nunc, Denmark) were coated with 50 µl of SLA (10 µg/ml) in PBS buffer (0.01M, pH= 7.3) and serial dilutions of serum at 4 °C for overnight. The plates

were treated with HRP-rabbit anti-mouse IgG isotype according to the manufacturer's instructions (Invitrogen Inc., USA). Optical density (OD) was determined at 450 nm using 630 nm as the reference wavelength [42].

Cytokine ELISA

The level of IL-4 and IFN-γ were determined in culture supernatants at 72-h using ELISA method. Briefly, three mice from each group were sacrificed at week 3 after the last booster, at the same time when the mice were challenged. The spleens were aseptically removed and mononuclear cells were isolated using Ficoll-Hypaque (Biogene, Iran) density centrifugation method [42]. The cells were washed and resuspended in complete medium (RPMI 1640-FCS) and seeded at 2 × 10^6/mL in 96-well flat-bottom plates (Nunc, Denmark). Then, the spleen cells were cultured and stimulated with SLA (10 µg/mL) or as control with medium alone and incubated at 37ÏC with 5% CO2 for 72 h. The culture supernatants were collected and the level of IFN-γ and IL-4 were titrated using ELISA method according to the manufacturer's instructions (MabTech, Sweden).

Flow cytometry analysis

Spleenocytes were isolated 2 weeks after the last booster, and stained for intracellular cytokine IFN-γ (anti- IFN-γ –FITC) and IL-4 (anti-IL-4-FITC) according to BD protocols Cytofix/Cytoperm™ Plus Fixation/ Permeabilization Kit. Cell surface antigens CD4 and CD8 were stained. The cells were fixed and permeabilized, and finally secreted intracellular cytokines were stained and then analyzed on a FACS Calibur (BD Biosciences).

Statistical analysis

Data were recorded in GraphPad Prism software and analyzed. One-way ANOVA statistical test was used to assess the significance of the differences among the various groups. The mean and standard deviation of all experiments were determined. In case of significant

RESULTS

Characterization of ISCOMATRIX

The mean diameter and surface charge of formulation were calculated by zeta sizer (Malvern, UK) for each preparation and are shown in Table1. ISCOMATRIX were homogeneous in the size, the mean for positively and negatively charged ISCOMATRIX were 93.7 ± 10.8 and 110.8 ± 22 nm (n = 3), respectively. The overall poly dispersity index (PDI) of around 0.2 indicates that all different formulations are fairly homogenous. Zeta potential in positively and negatively charged ISCOMATRIX was 37.2 ± 5.4 and -14 ± 2.8 (mv), respectively. Detection of SLA in different formulations containing SLA was performed using SDS-PAGE electrophoresis (Fig1). SDS-PAGE analysis of SLA revealed several protein bands with molecular weight ranges from 10 to 70 kDa. Result of transmission electron microscopy (TEM) showed that hollow cage-like structures which are considered as the typical morphology of ISCOM matrices and the size of ISCOMATRIX structures was in the range of 50-80 nm (Fig2).

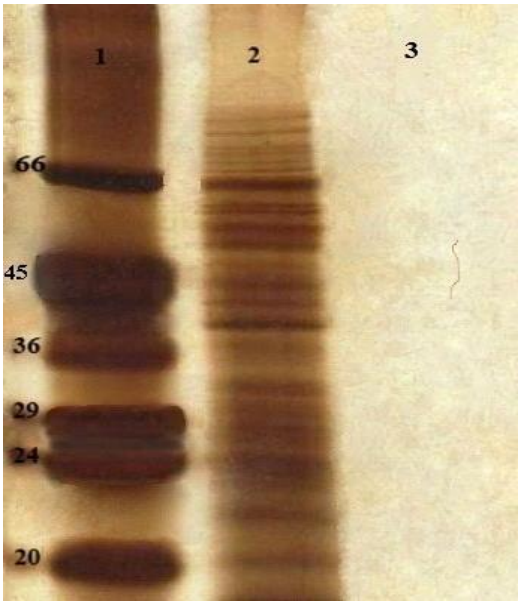
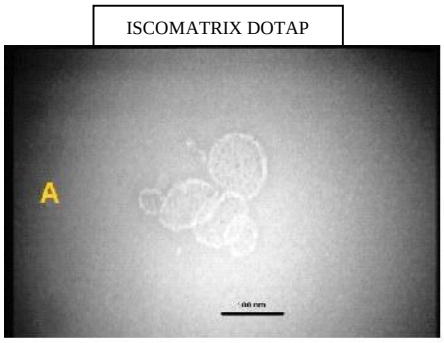


Fig. 1. SDS-PAGE analysis of SLA alone and ISCOMATRIX. Lane 1, Low-range protein standard (Sigma, USA); Lane 2, SLA (10 µg); Lane 3, ISCOMATRIX without SLA



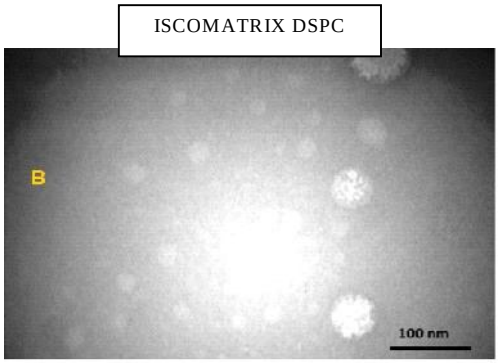


Fig. 2. Photographs taken by transmission electron microscopy (TEM) from the ISCOMATRIX DSPC (A) and ISCOMATRIX DOTAP (B)

Table 1. Particle size distribution, polydispersity index (PDI) and zeta potential of various Iscom Matrix formulations (mean ± SD, n = 3)

Formulation	Size(nm)	PDI	Zeta potential (mv)
ISCOMATRIX DOTAP	93.7 ± 10.8	0.3 ± 0.06	37.2 ± 5.4
ISCOMATRIX DSPC	110.8 ± 22	0.29 ± 0.07	-14 ± 2.8

Challenge results

To investigate the lesion development and progress, the immunized mice with L. major promastigotes were recorded weekly by measurement of footpad thickness (Fig 3). Footpad thickness progressed similarly in all groups of mice up to two weeks after the challenge. The lesion size progressed in all mice immunized with different formulations from week 3 post infections. Footpad swelling in all groups was progressed continuously and no protection was observed in any groups. The smallest footpad swelling was seen in the group of mice immunized with negatively charged ISCOMATRIX DSPC compared with all other groups. But, there was no significant difference between the group of mice immunized with positively charged, negatively charged ISCOMATRIX footpad swelling in comparison with the group of mice received buffer. In all groups, the footpad swelling reached a plateau after 5 weeks but the disease progressed by metastasis to other organs and some of the mice lost their foot.

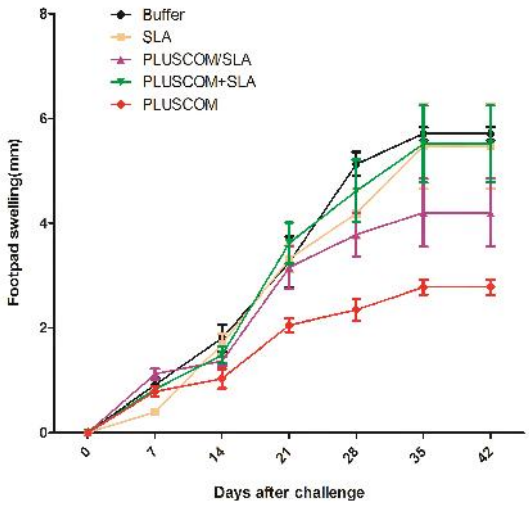
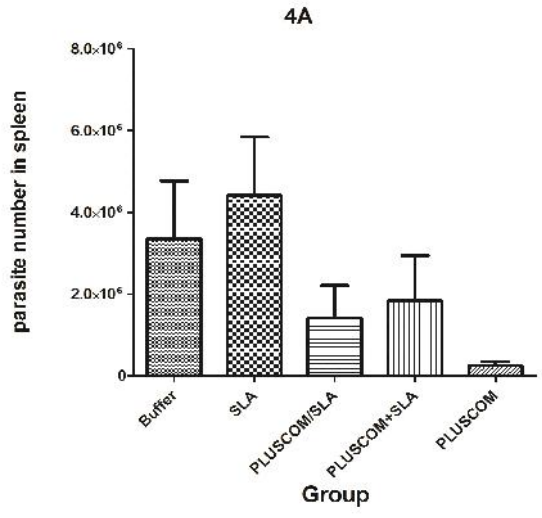


Fig. 3. Footpad swelling in BALB/c mice immunized SC, three times in 3-week intervals, with SLA, ISCOMATRIX DOTAP, ISCOMATRIX DSPC and buffer alone. The footpad thickness of each mouse was measured on both footpads for 42 days. Each point represents the average increase in footpad thickness ± SEM (n=7)

Splenic parasite burden after challenge

The number of viable L. major at day 42 after challenge was quantified in spleen of different mice groups (Fig 4A). Results showed that the group of mice immunized with positively charged showed the least parasite burden, but was not significant ($p < 0.05$) compared to the other groups.



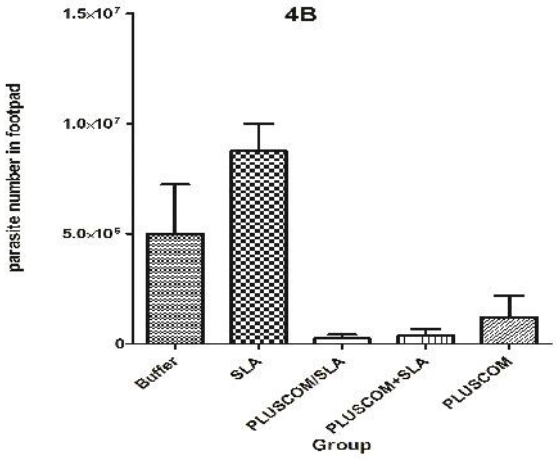


Fig. 4. Results of parasite burden in mice immunized subcutaneously (SC), with SLA, ISCOMATRIX DOTAP, ISCOMATRIX DSPC or buffer alone after challenge with L. major promastigotes, spleen parasite burden (4A) and footpad parasite burden (4B)

Parasite burden in foot after challenge

The number of viable L. major was quantified in the infected foot-pad of different groups of mice at day 42 after challenge (Fig 4B). As showed, the group of mice immunized with ISCOMATRIX DOTAP showed the least parasite burden compared with the other groups. There was no significant ($p < 0.05$) difference between mice immunized with positively charged and those received negative charged ISCOMATRIX and also no significant difference in the group of mice received buffer as a control.

Antibody results

To determine the type of immune response generated, the serum samples were collected prior and post-challenge and anti Leishmania IgG isotypes were tested (Fig 5 and 6). As shown in Fig 5, before Challenge there was a significantly ($P < 0.001$) higher level of IgG2a, IgG1,IgG antibodies in the sera of mice immunized with negatively charged or positively charged ISCOMATRIX compared to the control group. In terms of after challenge, the sera of mice immunized with positively or negative charged ISCOMATRIX showed significantly ($P < 0.05$) the highest level of specific IgG1 antibody isotypes against SLA antigen compared with the other groups. The level of IgG2a antibody in the sera of mice immunized with positively or negatively charged ISCOMATRIX was significantly ($P < 0.01$) higher than the group of mice immunized with SLA or the control group. Also Fig 6 shows, after challenge, a significantly ($P < 0.05$) high level of IgG total antibody in the sera of mice immunized with negatively charged ISCOMATRIX was seen compared with the other groups.

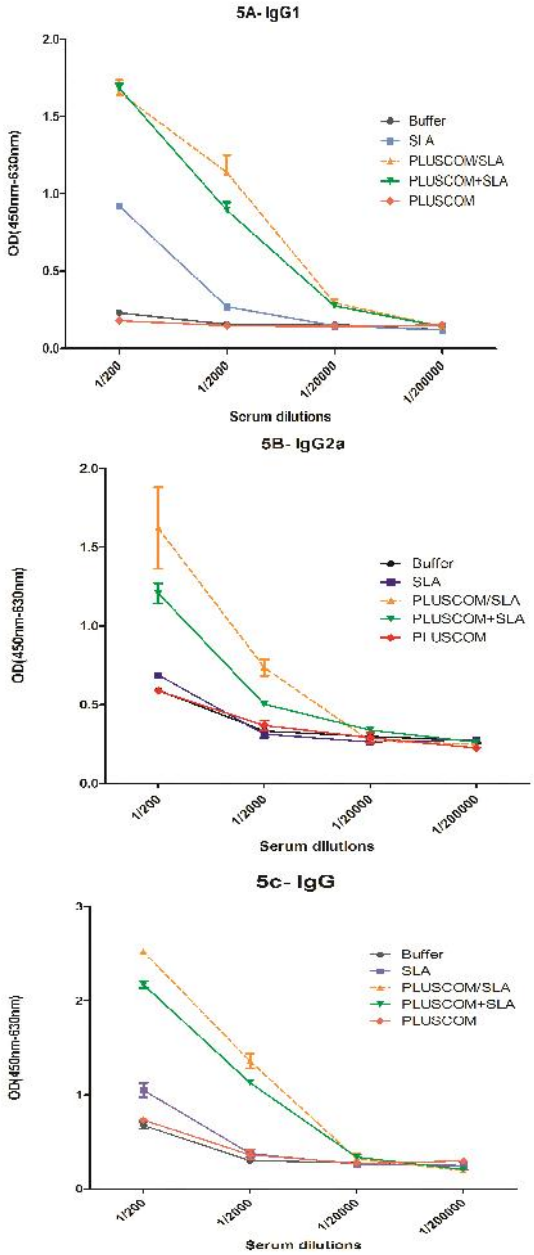


Fig. 5. The levels of anti-SLA specific IgG1, IgG2a, and IgG antibodies based on mean absorbance in sera of BALB/c mice. Mice immunized SC, three times in 3-week intervals, with SLA, ISCOMATRIX DOTAP, ISCOMATRIX DSPC or buffer alone. Blood samples were collected from the mice 2 weeks after the last booster (5A, 5B, 5C). The assays were performed using ELISA method for each serum sample. Values are the mean ± SD

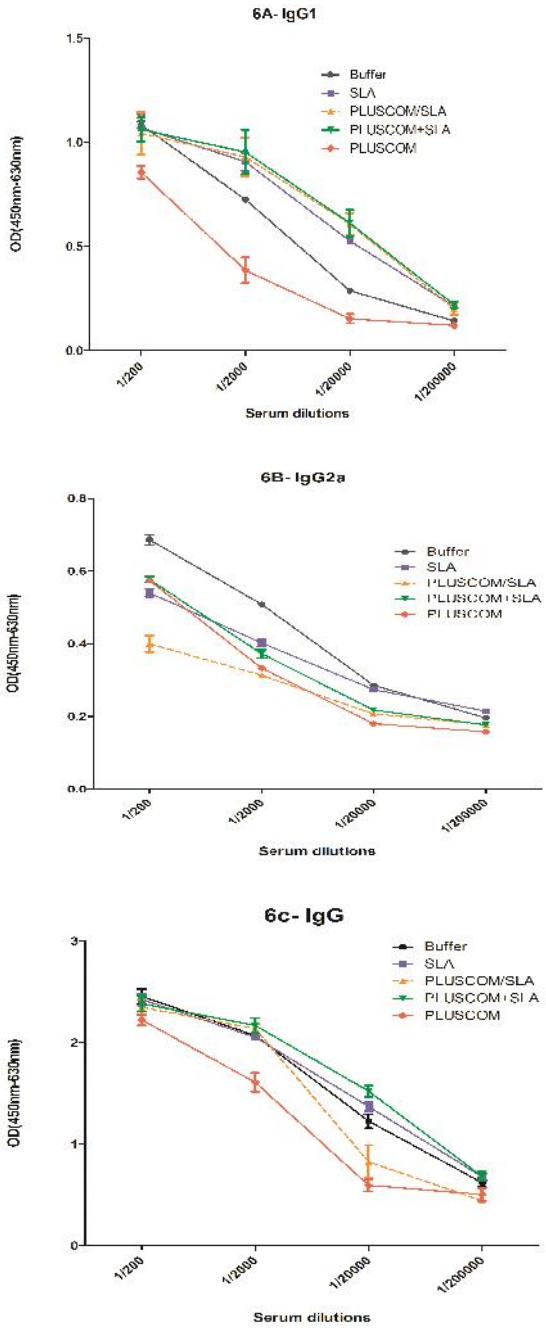
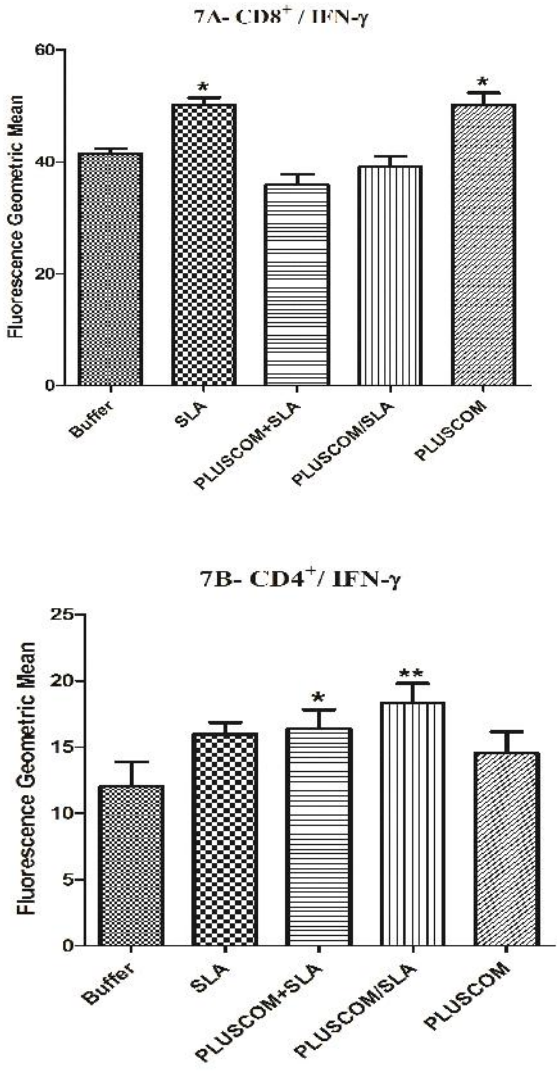


Fig. 6. The levels of anti-SLA specific IgG1, IgG2a, and IgG antibodies based on mean absorbance in sera of BALB/c mice. Mice immunized SC, three times in 3-week intervals, with SLA, ISCOMATRIX DOTAP, ISCOMATRIX DSPC or buffer alone. Blood samples were collected from the mice 6 weeks after challenge (6A, 6B, 6C). The assays were performed using ELISA method for each serum sample. Values are the mean ± SD

In vitro cytokine production by splenocytes

The day before challenge, spleens of mice were isolated and cultured. The results showed that the significantly ($P < 0.001$) highest level of IFN-γ was detected in the cell supernatant of mice immunized with negatively charged ISCOMATRIX in comparison with the other group of mice (Fig 7A).

The least amount of IL-4 was detected in the splenocytes of group of mice immunized with positively charged ISCOMATRIX. However, there was a significant difference in the level of IL-4 between mice immunized with negatively charged ISCOMATRIX ($P < 0.001$) in comparison with the control group of mice (Fig 7B).



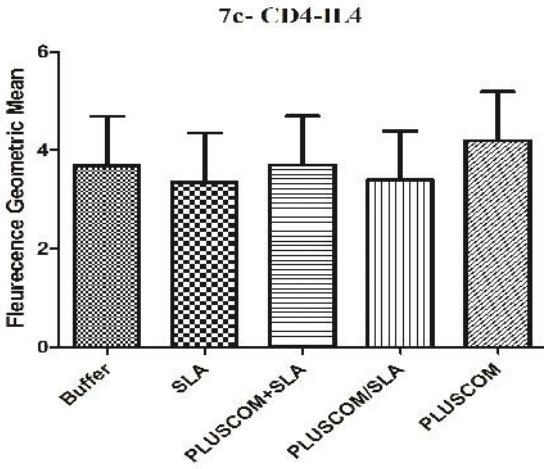


Fig. 7. Mice immunized with SLA, ISCOMATRIX DOTAP and ISCOMATRIX DSPC or buffer alone. Production of IFN-γ (7A) and IL-4 (7B) were assessed by sandwich ELISA. The data indicate the mean ± S.E.M, (n = 3). * (P<0.05) and *** (P<0.001) denote significant difference from buffer and all other formulations, respectively

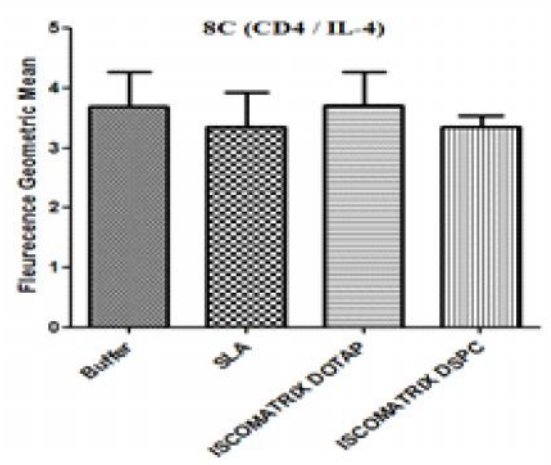
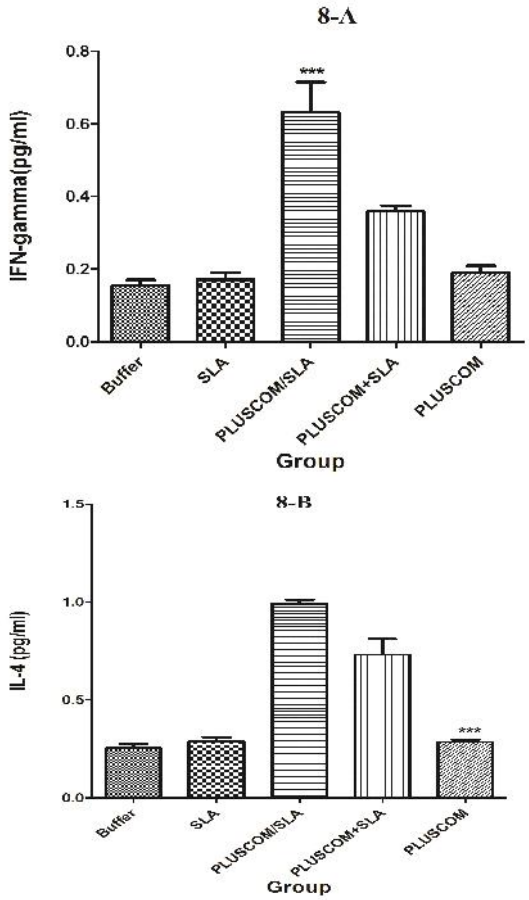


Fig. 8. After last booster, splenocytes were isolated and restimulated, then stained for surface CD8, CD4 and intracellular IFN-γ and IL-4. Splenocytes were gated by side vs forward scatter light followed by staining with CD8Pe-cy5 and CD4Pe-cy5. Plots show log fluorescence intensity for IFN-γ- FITC and IL-4-PE. The data indicate the mean ± S.E.M, (n = 3). *(P<0.05) and ** (P<0.01) denote significant difference from buffer and all other formulations, respectively



Flow cytometery results

To determine the antigen-specific T cell responses, after the last booster, splenocytes were isolated in different groups of mice. Extracellular staining was used for CD4 and CD8 surface markers and intracellular cytokine staining was used for IFN-γ and IL-4 cytokines followed by flow cytometery analyses. As shown in Fig 8, the results showed SLA and ISCOMATRIX DOTAP formulations induced a significantly ($p<0.05$) higher level production of IFN-γ in $CD4^+$ lymphocytes which represented a higher number of IFN-γ producing cells in $CD4^+$ population in comparison with other groups.

The frequency of $CD8^+$/IFN-γ cells in the group of mice immunized with SLA was significantly ($p<0.01$) greater than that of other groups.

However flow cytometric results also showed IL-4 production in CD^+4 cells that implies T cell-dependent humoral immunity, were not induced significantly in all groups compared with buffer.

DISCUSSION

Successful vaccination strategies for Leishmania have relied on presentation of antigen with appropriate

adjuvants to the host immune system to stimulate effective cell-mediated immune responses. Phagocytosis and presentation of nanoparticle-associated antigens have been shown to be strongly influenced by the chemical and physical nature of particles [43].

Interactions between particulate formulations and cells in general depend on particle characteristics such as size and surface properties, including surface charge and hydrophobicity [44].

There are some controversies regarding the surface charge of nanoparticles and type of generated immune response. For example, negatively charged liposomes are taken up by APC more than either neutral or positively charged vesicles [45], whereas other reports have shown that positively charged liposomes are taken up more effectively than negatively one [46,47]. In the present study, we addressed the comparison of ISCOMATRIX formulations using different charges on the type of generated immune response against CL in BALB/c mice. To prepare, net positively or negatively charged ISCOMATRIX, DOTAP was replaced by DSPC, respectively.

The lesion size, splenic parasite burden, evaluation of Th1 cytokine (IFN-γ) and Th2 cytokine (IL-4), and titration of IgG isotypes were carried out to assess the type of generated immune response and extent of protection. The results of the current study showed that mixed Th1/Th2 immune response was seen by positively or negative charged ISCOMATRIX. The cytokine assay results showed IFN-γ secretion was detected in the cell supernatant of mice immunized with negative charged ISCOMATRIX in comparison with the other group of mice. Interestingly, the highest amount of IL-4 was detected in the splenocytes of mice immunized with ISCOMATRIX DSPC.

According to our results, we concluded that the charge of lipids do not have a role in induced immune response.

It seems that Ags mixd with ISCOMATRIX adjuvant access both the class II MHC processing pathway for presentation to $CD4^+$ T cells and generation of B-cell responses as well as the cytosol for access to the class I MHC processing pathway for presentation to $CD8^+$ T cells [48, 49]. ISCOMATRIX vaccines are potent inducers of both $CD4^+$ and $CD8^+$ T-cell responses for a wide variety of Ags, including naturally occurring immunogens, recombinant proteins, peptides [51] and a linear array of class I MHC epitopes referred to as a polytope vaccine [52]. Polakos and colleagues demonstrated that a HCV core ISCOMATRIX vaccine induced strong $CD4^+$ and $CD8^+$ T-cell responses in rhesus macaques [53].

In this study, ISCOMATRIX DOTAP formulations generate $CD4^+$/ IFN-γ cells more than buffer group. The ISCOMATRIX adjuvant is well suited for prophylactic vaccines, where the induction of strong, long-lived, neutralizing Ab responses is required. The induction of a balanced Th1/Th2 cytokine response and generation of Abs of all immunoglobulin G (IgG) isotypes (including IgG1 and IgG2a in mice) can potentially facilitate a broader range of Ab-mediated effector mechanisms (for example, complement activation, viral neutralization, Ab-dependent cell-mediated cellular cytotoxicity, opsonization and phagocytosis [54].

Drane results indicated that HCV Core ISCOMATRIX ™ vaccine induce a high titer HCV-specific antibody response (IgG) [56]. Similarly, our results showed that in all cases, antibody titers (IgG, IgG2a, and IgG1) of negatively charged ISCOMATRIX are higher than buffer group.

The results of this study and other studies concluded that Quil A adjuvant has an important role in the immune response. Because Quil A is a heterogenous mixture of saponins when analyzed using RP-HPLC (Fig 1) [57], it is possible that the various componentsmay produce different levels of adjuvanticity and toxicity that could be exploited to produce useful adjuvants for human vaccines.

Therefore, the purification and structure–function relationships of adjuvant-active saponins have been the subject of interest.

Quil A is a heterogeneous mixture of more than 20 components [58]. Different saponins of Quil A such as QS-7, QS-17, QS-18 and QS-21 have also been differ immune stimulating properties.

The different characteristics of Quil A might have different effects on its properties such as interaction with antigen presenting cells (APC), activation of CTL, stimulation of T helper (Th) cell subsets and the type of immune response [59]. Using a specific derivative of Quil A, such as QS-21, may improve the efficacy of ISCOMATRIX vaccines for full protection in Leishmania infections.

CONCLUSION

In summary, the current results showed that ISCOMATRIX formulations with different charges induced a mixed Th1/Th2 response that was not protective in leishmaniasis.

ACKNOWLEDGMENTS

The financial support of the Nanotechnology Research Center Mashhad University of Medical Sciences are gratefully acknowledged. This study was part of Ph.D. dissertation of AM that was completed in Nanotechnology Research Center, MUMS, Iran.

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How to cite this article:
Mehravaran A, Jaafari MR, Jalali SA, Khamesipour A, Tafaghodi M, Hojatizade M, Badiee A . The role of surface charge of ISCOMATRIX nanoparticles on the type of immune response generated against Leishmaniasis in BALB/c mice. Nanomed. J., 2015; 2(4): 249-260.