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REVIEW

GM3-containing nanoparticles in immunosuppressed hosts: Effect on myeloid-derived suppressor cells

Audry Fernández, Liliana Oliver, Rydell Alvarez, Luis E Fernández, Circe Mesa

Audry Fernández, Liliana Oliver, Rydell Alvarez, Circe Mesa, Immunobiology Division, Center of Molecular Immunology, Havana 11600, Cuba
Luis E Fernández, Innovation Division, Center of Molecular Immunology, Havana 11600, Cuba
Author contributions: Fernández A, Oliver L, Alvarez R, Fernández LE and Mesa C drafted the review and wrote the paper.
Supported by Center of Molecular Immunology
Correspondence to: Circe Mesa, PhD, Director of Immunobiology Division, Center of Molecular Immunology, 216 St. and 15th Avenue, Atabey, Playa, PO Box 16040, Havana 11600, Cuba. circe@cim.sld.cu
Telephone: +53-7-2143161 Fax: +53-7-2720644
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Abstract

Cancer vaccines to date have not broadly achieved a significant impact on the overall survival of patients. The negative effect on the immune system of the tumor itself and conventional anti-tumor treatments such as chemotherapy is, undoubtedly, a key reason for these disappointing results. Myeloid-derived suppressor cells (MDSCs) are considered a central node of the immunosuppressive network associated with tumors. These cells inhibit the effector function of natural killer and $CD8^+$ T cells, expand regulatory T cells and can differentiate into tumor-associated macrophages within the tumor microenvironment. Thus, overcoming the suppressive effects of MDSCs is likely to be critical for cancer immunotherapy to generate effective anti-tumor immune responses. However, the capacity of cancer vaccines and particularly their adjuvants to overcome this inhibitory population has not been well characterized. Very small size proteoliposomes (VSSP) is a nanoparticulated adjuvant specifically designed to be formulated with vaccines used in the treatment of immunocompromised patients. This adjuvant contains immunostimulatory bacterial signals together with GM3 ganglioside. VSSP promotes dendritic cell maturation, antigen cross-presentation to $CD8^+$ T cells, Th1 polarization, and enhances $CD8^+$ T cell response in tumor-free mice. Currently, four cancer vaccines using VSSP as the adjuvant are in Phase Ⅰ and Ⅱ clinical trials. In this review, we summarize our work characterizing the unique ability of VSSP to stimulate antigen-specific $CD8^+$ T cell responses in two immunocompromised scenarios; in tumor-bearing mice and during chemotherapy-induced leukopenia. Particular emphasis has been placed on the interaction of these nanoparticles with MDSCs, as well as comparison with other cancer vaccine adjuvants currently in preclinical or clinical studies.

Key words: Very small size proteoliposomes; Adjuvants; Tumors; Myeloid-derived suppressor cells; Leukopenia; Chemotherapy

Core tip: Very small size proteoliposomes (VSSP) is a nanoparticulated adjuvant being used in the formulation of several cancer vaccines that are currently in clinical trials. In this review we summarize the unique ability of VSSP to stimulate antigen-specific $CD8^+$ T cell responses in tumor-bearing mice and in mice with chemotherapy-induced leukopenia, both immunosuppressive scenarios frequently found in cancer patients. As a possible mechanism of this efficacy, we have focused on the modulation of myeloid-derived suppressor cells (MDSCs) by these nanoparticles, in the context of the current knowledge about the interaction of cancer vaccine adjuvants with MDSCs.

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INTRODUCTION

The central importance and complexity of the interactions between tumors and the immune system has only recently been recognized, with rapidly expanding investigations in the last decade. Tumors are not only shaped by the immune system[1,2] but actively induce impairment of antigen-presenting cells (APCs) as well as effector T lymphocytes[3,4], contributing significantly to both tumor progression and metastasis. One of the key cellular mediators of tumor-induced immunosuppression are myeloid-derived suppressor cells (MDSCs), which not only are the manifestation of the myeloid differentiation block that causes loss of mature APCs, but also actively and directly inhibit the lytic activity of both $CD8^+$ T cells[5,6] and NK cells[7].

MDSCs are currently thought of as a heterogeneous population of immature myeloid cells with suppressive activity. In mice these cells are routinely identified by the co-expression of CD11b and Gr1 markers. More recently, two subpopulations of MDSCs have been identified with different phenotypes and mechanisms of suppression: monocytic (Mo-MDSCs) and granulocytic (G-MDSCs)[8-11]. In tumor-bearing mice, as well as in cancer patients, the G-MDSCs constitute 70%-80% of overall MDSCs, whereas Mo-MDSCs represent only 20%-30%[11-14]. Mo-MDSCs ($CD11b^+Ly6C^{hi}Ly6G^-$) are highly immunosuppressive and exert their suppression *via* antigen-independent mechanisms[15-18]. In comparison, G-MDSCs ($CD11b^+Ly6C^{lo}Ly6G^+$) are moderately immunosuppressive, release reactive oxygen species (ROS) and require antigen-specific interaction with T cells to induce tolerance[9,11,19,20]. Several mechanisms of MDSC-mediated suppression have been described and are extensively detailed in other reviews[3,21]. Among these, the depletion of L-arginine, production of nitric oxide (NO) and generation of ROS/reactive nitrogen species have been linked to the overexpression of arginase 1 (ARG1), inducible nitric oxide synthase (NOS2) and NADPH oxidase[3,13,22]. MDSCs are also able to expand regulatory T cells (Tregs) populations[23,24] and can differentiate into tumor-associated macrophages within the tumor microenvironment[25,26]-both regulatory populations that play an important role in tumor-induced immunosuppression. Recent findings suggest that MDSCs can also facilitate tumor-progression and metastasis by increasing angiogenesis[27,28], *via* secretion of matrix metallopeptidases[29,30] and by aiding in the formation of the metastatic niche[27,31].

Given the pro-tumor importance of MDSCs, many efforts have been undertaken to find drugs capable of reducing the number of circulating MDSCs, abrogate MDSCs suppressive function or differentiate these cells into mature APCs. For instance, it has been demonstrated that 25-hydroxy vitamin D3 and all-trans retinoic acid reduce the frequency of MDSCs by inducing their differentiation towards $HLA\text{-}DR^+$ cells and dendritic cells (DCs), respectively, in patients with advance head and neck squamous cell carcinoma and metastatic renal cell carcinoma (RCC)[32-34]. Sunitinib, a pan-receptor tyrosine kinase inhibitor, and chemotherapeutic agents (taxanes, gemcitabine and 5-fluorouracil) also decrease circulating MDSCs in patients with RCC, melanoma, pancreatic and esophagogastric cancer[35,36]. Finally, the phosphodiesterase-5 inhibitor sildenafil diminishes the suppressive function of human MDSCs[37].

Although the pharmacological modulation of MDSCs represents a potentially important strategy for cancer treatment, none of these drugs detailed above have thus far improved the clinical outcome in cancer patients. These data suggest that inhibiting MDSCs alone (unlike the T cell checkpoint inhibitors) is not sufficient to achieve an effective anti-tumor response, and that combination with strategies to specifically activate immune responses against the cancer are needed. However, most cancer vaccines have not shown significant objective responses in clinical trials. But, the unimpressive clinical impact of active immunotherapy in cancer patients may be in turn tied to the immunosuppressive environment generated by tumors[3,4,21] as well as the aggressive chemotherapeutic treatments used in patients, which frequently induce leukopenia[38-40]. Thus, the combination of cancer vaccines with agents interfering with MDSCs number/function may be an effective approach to generate fully functional tumor-specific immune effectors. Even more desirable would be to find agents that are capable of simultaneously activating tumor-specific effector cells, inhibiting the suppressive function of MDSCs, and diminishing leukopenic period after chemotherapy. As detailed below, these are all properties of the VSSP adjuvant.

Adjuvants are critical but largely unappreciated components of vaccine formulations, necessary to potentiate the immune response specific for the nominal antigen. This is particularly important in cancer, where the vaccine antigen is often a self protein for which self-tolerance needs to be broken. In recent years the interaction of adjuvants with regulatory cells, and particularly MDSCs, have begun to be study[41-45]. This field is still in its infancy however, and there is only strong evidence for the modulation of tumor-induced MDSCs by synthetic oligodeoxynucleotides containing unmethylated CpG motifs (CpG)[44], formalin-inactivated Herpes Simplex Virus[43] and VSSP[42], while indirect evidence suggests that other adjuvants may expand MDSCs once inoculated in the hosts. Therefore, the selection of suitable adjuvants for cancer vaccines is a very complex matter, and needs to be based in the ability to overcome the immunosuppression generated by tumors and chemotherapy. In this review we summarize the immunomodulatory properties of VSSP, a novel adjuvant for cancer immunotherapy.

GENERAL PROPERTIES OF VSSP

VSSP is a nanoparticulated adjuvant obtained through the hydrophobic incorporation of the GM3 ganglioside into outer membrane vesicles (OMPs) from *Neisseria meningitidis*[46]. It has been shown that VSSP contains TLR4

Table 1 Modulation caused by very small size proteoliposomes on different immune cell populations

Immune cell	Effect of VSSP	Ref.
DCs	Increases costimulation and MHCII expression	[47]
	Enhances production of IL-12, IL-6, IL-18, IL-1β and reduces secretion of IL-10	[47,48]
	Induces Th1-polarizing capacity	[47]
	Facilitates cross-presentation of protein antigens	[50]
MDSCs	Expands poorly suppressive MDSCs	[42]
	Reduces the suppressive function of tumor-induced MDSCs	[42]
	Impairs migration of tumor-induced MDSCs towards the tumor microenvironment	[42]
	Promotes differentiation of tumor-induced MDSCs into mature DCs	[42,59]
	Reduces the suppressive function of MDSCs generated during chemotherapy-induced leukopenia	[62]
$CD4^+$ T cells	Induces Th1 polarization	[47]
CTL	Potentiates CTL responses in healthy mice. Primary expansion independent of $CD4^+$ T cell help	[50]
	Generates similar CTL responses in tumor-free and tumor-bearing mice	[42]
	Increases $CD8^+$ T cell counts, with memory phenotype, and protects CTL response in leukopenic mice	[62]

VSSP: Very small size proteoliposomes; MDSCs: Myeloid-derived suppressor cells; DCs: Dendritic cells; CTL: Cytotoxic T lymphocytes.

and TLR2 ligands, which play an important role in the immunomodulatory properties of this compound[47,48]. Immunization of mice, monkeys and humans with VSSP generated IgM and IgG antibodies specific for both GM3 and OMPs[46,49]. This adjuvant also induced DC maturation, as evidenced by the increased expression of MHCII and CD40, CD80 and CD86 costimulatory molecules (Table 1)[47]. Additionally, VSSP-treated DCs secreted inflammatory cytokines such as IL-12p40/70 and IL-6[47]. DCs from healthy donors treated *in vitro* with VSSP produced not only higher levels of IL-6 but also decreased amount of IL-10, in comparison to lipopolysaccharide [LPS, the prototypic TLR4 agonist (Table 1)][48]. Experiments with antigen-specific transgenic T cells demonstrated that VSSP-treated DCs induced a Th1 phenotype in stimulated naïve $CD4^+$ T cells[47]. Furthermore, VSSP expanded $CD8^+$ T cells specific for the co-injected antigen and promoted an effective *in vivo* cytotoxic T lymphocytes (CTL) response[50]. In the latter case, $CD8^+$ T cell activation was mediated by the cross-presentation of exogenous antigens and did not require help from $CD4^+$ T cells (Table 1)[50].

More recently, we have found that VSSP treatment of naive mice (without a vaccine antigen) significantly increased the frequency of splenic $CD11b^+Gr1^+$ cells[42]. However, these $CD11b^+Gr1^+$ cells were poorly suppressive on both antigen-specific and allogeneic CTL assays (Table 1). The residual suppressive capacity of VSSP-derived MDSCs depended on NOS but not ARG, which was associated with a significant increase of NOS3 enzyme. Although VSSP contains TLR2 and TLR4 ligands, the interaction of these particles with the immune system appears to be more complex than can be explained by just TLR activation. For example, OMPs containing the same TLR ligands induced a significantly lower expansion of $CD11b^+Gr1^+$ cells than did VSSP, indicating that the presence of the GM3 ganglioside is also relevant for the immunomodulatory properties of this compound.

VSSP-induced expansion of MDSC numbers is not entirely unexpected, as MDSCs have also been reported to accumulate in mice treated with granulocyte and macrophage colony-stimulating factor (GM-CSF)[51,52], LPS[41], CpG[53], complete Freund's adjuvant[45] and Bacillus Calmette-Guérin from *Mycobacterium bovis*[54]. Similar MDSCs expansion has been described for other conditions involving major inflammatory responses, such as superantigen vaccination[55], polymicrobial sepsis[56], after burn[57] and traumatic injuries[58]. These findings are consistent with a physiological role of MDSCs as a counterbalancing mechanism to inflammation, preventing collateral damage to the tissue caused by activated T cells once the "dangerous" antigen has been eliminated.

EFFECT OF VSSP ON TUMOR-BEARING IMMUNOCOMPROMISED HOSTS

The effect of VSSP on the phenotype, suppressive function and differentiation status of tumor-induced MDSCs has been evaluated in mice bearing C26GM, EL4, EG.7 and MCA203 tumors (Table 1)[42]. Splenic MDSCs derived from VSSP-treated tumor-bearing mice (MDSCs-T+V) contained a higher frequency of $CD11b^+Gr1^{hi}$ and $Ly6C^{lo}Ly6G^+$ G-MDSCs than untreated tumor-bearing counterparts (MDSCs-T). In addition, IL-4Rα is downregulated on MDSCs-T+V, and these cells showed an increase of the homing molecule CD62L. Consistent with our *in vitro* studies, the suppressive function of tumor-induced splenic MDSCs was significantly reduced when VSSP is given *in vivo*. Several different findings support this effect of VSSP. First, MDSCs-T+V were unable to suppress the hemagglutinin (HA) peptide-specific proliferation of $CD8^+$ T cells from CL4 TCR transgenic mice, in the same experimental setting where equal number of MDSCs-T were significantly inhibitory. *In vitro* ^{51}Cr release CTL assays demonstrated that, as expected, MDSCs-T completely suppressed both antigen-specific and alloantigen-specific lytic activity of $CD8^+$ T cells. In contrast, MDSCs-T+V isolated from EL4 and C26GM tumor-bearing mice only marginally affected the generation of the CTL.

The effect of VSSP on MDSCs *in vivo* was further examined in adoptive transfer experiments. In the first approach, MDSCs-T and MDSC-T+V were adoptively transferred into $CD45.1^+$ B6 congenic mice, which previously received the transference of ovalbumin (OVA)-specific $CD8^+$ T cells from OTI transgenic mice, and vaccinated with the immunodominant $OVA_{257-264}$ (SIINFEKL) peptide emulsified in incomplete Freund's adju-

vant (IFA). Similar frequencies of IFN-γ$^+$ antigen-specific CD8$^+$ T cells were found in recipient mice transferred with MDSCs-T+V compared to control mice receiving no MDSCs, whereas transfer of MDSCs-T significantly impaired the activation of OTI lymphocytes. Additional experiments were performed to compare VSSP with other adjuvants or well-established vaccination systems. On this regard, we found that VSSP-based vaccines are more efficient than vaccination with DCs or vaccines employing the adjuvant polyinosinic:polycytidylic acid (polyI:C) in activating antigen-specific CTL responses in the presence of MDSCs-T. In fact, vaccination of BALB/c mice, which had been adoptively transferred with both congenic antigen-specific CD8$^+$ T cells and MDSCs-T, with HA peptide in VSSP adjuvant prevented the MDSCs-T-mediated suppression of CD8$^+$ T cell responses that was observed in mice vaccinated with HA-pulsed DCs. Also congenic OTI CD8$^+$ T cells transferred to EG.7 tumor-bearing mice produce IFN-γ in response to VSSP admixed with SIINFEKL peptide- but not to a vaccine consisting of SIINKEKL-pulsed DCs. Importantly, the OVA-specific *in vivo* CTL response generated in mice with EL4 tumors by the administration of OVA/VSSP was comparable to that observed in tumor-free mice, whereas vaccination with OVA/polyI:C was unable to overcome the tumor-induced impairment of the CTL response.

In addition to TCR transgenic T cell responses to a model antigen, we have found that VSSP blunts MDSC-mediated suppression of endogenous T cell responses to native tumor antigen, by measuring the inhibition of tumor-specific CD8$^+$ T cells by MDSCs in an ELISPOT assay. CD8$^+$ T cells isolated from MCA203 tumor-bearing mice did not release IFN-γ when stimulated with MCA203 tumor cells, irrespective of the presence of MDSCs. In contrast, a significant frequency of CD8$^+$ T cells derived from VSSP-treated tumor-bearing mice were activated by tumor cells and produced IFN-γ, even when MDSCs-T+V were added to the culture. Importantly, MDSCs-T maintained their ability to suppress tumor-specific CTL in this experiment.

Within the tumor microenvironment itself, VSSP treatment did not change the phenotype and functional capacity of CD11b$^+$ sorted MDSCs. However, adoptively transferred congenic MDSCs-T had a reduced ability to infiltrate tumors in EL4 tumor-bearing mice treated with VSSP. More importantly, in these VSSP-treated mice, tumor-infiltrating transferred MDSCs-T were more differentiated into CD11c$^+$MHCII$^+$CD11b$^-$ phenotype characteristic of DCs, and did not differentiate towards MHCII$^+$F4/80$^+$ macrophages. A similar differentiation pattern was observed *in vivo* in the spleen and lymph nodes from VSSP-inoculated tumor-bearing mice. In a more recent work, it was demonstrated that *in vitro* treatment with VSSP of tumor-induced MDSCs was sufficient to differentiate this immature population towards phenotypically mature DCs and, more importantly, causes the loss of their suppressive function[59]. Since VSSP contains a TLR4 ligand, a comparison with LPS was done in the same experimental setting. Interestingly, incubation with LPS fails to differentiate tumor-induced MDSCs into DCs and, consequently, these cells retain their inhibitory activity[59]. In agreement with these results, Greifenberg *et al*[60] have shown that incubation of bone marrow (BM)-derived MDSCs with the combination of LPS and IFN-γ increases NO secretion, enhancing the suppressive activity of these MDSCs and impairing their maturation into DCs. These findings further suggest that VSSP's effect on MDSCs is not a shared characteristic of all TLR4 agonists, but is a unique property of VSSP. Other authors have reported that TLR4 signaling is involved in the promotion of tumor growth associated with the recruitment of G-MDSCs, through the interaction with S100A9 protein[61]. VSSP also expands G-MDSCs subpopulation in tumor-bearing mice, however it also potentiates CTL responses and anti-tumor activity on those mice[42]. Therefore, the complexity of signals in the structure of VSSP (TLR2 agonist, GM3 ganglioside, *etc.*) likely makes these particles distinct from single TLR4 agonists. In fact, VSSP can induce activation of BM-derived DCs obtained from LPS hyporesponsive mice (C3H/HeJ)[47].

It has been shown in the literature that other adjuvants can also reduce the suppressive function of tumor-recruited MDSCs. For instance, intratumoral injection of CpG reduces the suppressive function of Mo-MDSCs and induces their differentiation towards macrophages with tumoricidal capability[44]. However, CpG does not modify G-MDSCs, and intratumoral injections in patients may be difficult to impossible. Formalin-inactivated Herpes Simplex Virus also decreases the suppressive function of MDSCs-T, but whether this adjuvant is able to differentiate MDSCs has not been addressed[43].

INFLUENCE OF VSSP ON CHEMOTHERAPY-ASSOCIATED IMMUNOSUPPRESSED HOSTS

The ability of VSSP to rescue the number and functionality of relevant immune populations on mice undergoing chemotherapy-induced leukopenia has been also tested (Table 1)[62]. The widely used chemotherapy agent cyclophosphamide (CY) was used to induce the leukopenic setting for these studies. In this model, VSSP accelerated the recovery of specific leukocytes population when administered in the early stages of leukopenia. Splenic CD4$^+$ and CD8$^+$ T cells (with a memory CD4$^+$CD44^{hi} and CD8$^+$CD44^{hi} phenotype) and CD11c$^+$CD11b$^+$ DCs were some of the populations most enhanced by VSSP in leukopenic mice. Interestingly, MDSCs were also significantly expanded. However, similar to what was seen in the tumor-mediated immunosuppression setting, MDSCs from leukopenic mice treated with VSSP showed a reduced capacity to suppress T cell responses, compared to CY-induced MDSCs (Table 1). Importantly, in the same experimental setting, we found that polyI:C treat-

ment induced none of the effects observed with VSSP inoculation.

The ability of VSSP to activate antigen-specific $CD8^+$ T cells was also tested in leukopenic mice. In this immunocompromised scenario, vaccination with a single dose of OVA/VSSP, at the time point corresponding to the lowest CD8 counts, induced significant antigen-specific CTL responses. In comparison, vaccination with three doses of OVA/polyI:C was not capable of inducing antigen-specific effector $CD8^+$ T cell activation. Furthermore, VSSP treatment of OVA/polyI:C vaccinated animals restored the dampened CTL responses in polyI:C-treated leukopenic mice, indicating that VSSP can function as an immunomodulator as well. This effect could be associated to the capacity of VSSP, different from polyI:C, to accelerate the recovery of effector $CD8^+$ memory T cells and to induce the expansion of DCs and less suppressive MDSCs.

Granulocyte colony-stimulating factor (G-CSF) is the standard growth factor used in the clinic to revert chemotherapy-induced leukopenia, but also has been reported to be a tumor-derived factor that induces MDSCs generation and recruitment[63]. Therefore we assessed whether treatment with recombinant G-CSF could restore the *in vivo* CTL response barely induced by OVA/polyI:C vaccine in CY-treated mice[62]. Administration of G-CSF has no impact in the impaired antigen-specific CTL response, possibly due to the expansion of MDSCs but also *via* G-CSF-induced Th2 responses[64] and the resulting differentiation of Tregs that may impair effector T lymphocyte proliferation[65]. However, when VSSP was given with G-CSF, the ability of VSSP to restore $CD8^+$ T cell function was not affected, which opens the possibility for their concomitant use in the clinic. Moreover, the functionality of MDSCs recruited in these experiments was additionally evaluated. As expected from previous reports, our data also demonstrated that, in leukopenic mice treated with G-CSF, the induced MDSCs were highly suppressive. Importantly, the concomitant treatment with VSSP dampened the inhibitory function of MDSCs expanded after G-CSF injection. To our knowledge, no other adjuvant has been tested in this immunosuppressive leukopenic scenario induced by chemotherapy.

ANTI-TUMOR ACTIVITY OF VSSP

Several pre-clinical studies support the anti-tumor efficacy of VSSP, whether used alone or in combination with other tumor-associated antigens different from the GM3 ganglioside. The combination of surgery and VSSP alone prevented tumor recurrence and improved survival in melanoma B16F10 tumor-bearing mice[66]. In a different tumor model, treatment of mice bearing MCA203 tumors with three doses of VSSP was sufficient to significantly delay tumor growth[42]. Of interest, GM3 ganglioside, an important component of VSSP, is highly expressed on both melanoma B16F10 and MCA203 sarcoma. Particularly in MCA203 tumor-bearing mice, treatment with VSSP alone caused a significant increase in the frequency of classical IFN-γ-producing $CD8^+$ T cells specific for MCA203 antigens, suggesting an antigen-spreading likely induced by the initial response against the GM3 ganglioside[42]. Moreover, VSSP-adjuvanted vaccines (both peptides and whole proteins) have shown anti-tumor activity. For instance, a vaccine containing the extracellular domain of murine epidermal growth factor receptor (EGFR) and VSSP has a potent anti-metastatic effect in the Lewis lung carcinoma model[67]. In a mouse model of cervical cancer induced by Human Papilloma Virus (HPV), the immunization with an E7-derived CTL peptide from HPV 16 mixed with VSSP induced regression of established tumors[68]. Therapeutic vaccination of EG.7 tumor-bearing mice with OVA or SIINFEKL peptide adjuvated in VSSP, but not SIINFEKL emulsified in IFA, caused a significant reduction of tumor growth[42]. However, VSSP administration alone to EL4 and C26GM tumor-bearing mice, with the same schedule associated with the inhibition of MDSCs suppressive function, does not delay tumor growth. One possible explanation for the absence of an anti-tumor effect of VSSP alone in these models is the lack of a tumor-associated antigen during treatment, and consequently, the absence of antigen-specific $CD8^+$ T cell activation. In fact, EL4 tumors express low levels of GM3 whereas an inappropriate exposure of this ganglioside on the surface of C26GM tumor cells has been observed[42]. Altogether, these data strongly suggest that the best induction of anti-tumor responses requires combining the abrogation of tumor-induced MDSCs with a specific stimulation of T lymphocytes, which can be successfully done by mixing a proper tumor-associated antigen with VSSP.

Finally, four therapeutic cancer vaccines employing VSSP as adjuvant are in clinical trials. An EGFR-based vaccine[67] is currently in Phase I clinical trials. A Phase I clinical trial in patients with advanced solid tumors using a formulation of a mutated vascular endothelial growth factor[69] and VSSP has been recently completed. In this trial, the most common adverse events were Grade 1 pain and erythema at injection site and Grade 1 fever [70]. Additionally, a gonadotropin releasing hormone-based vaccine[71] and a HPV-derived peptidic vaccine[72] are currently in Phase Ⅱ trials in prostate cancer patients and women with high-grade cervical intraepithelial neoplasia, respectively. Both vaccines have previously shown to be safe and immunogenic. The most frequent adverse event in patients receiving the HPV vaccine was local pain at the vaccination site, whereas fever, tremors and cramps were seen in few cases, but none exceeded Grade 1[72]. Another Phase I trial using VSSP alone in metastatic melanoma patients demonstrated the safety of this preparation even in the presence of Montanide ISA 51, with toxicity consisting of local reaction at the site of injection and mild fever and chills[49]. In this trial both humoral and cellular responses were induced by the VSSP treatment. Additionally, an ongoing physician-lead trial is evaluat-

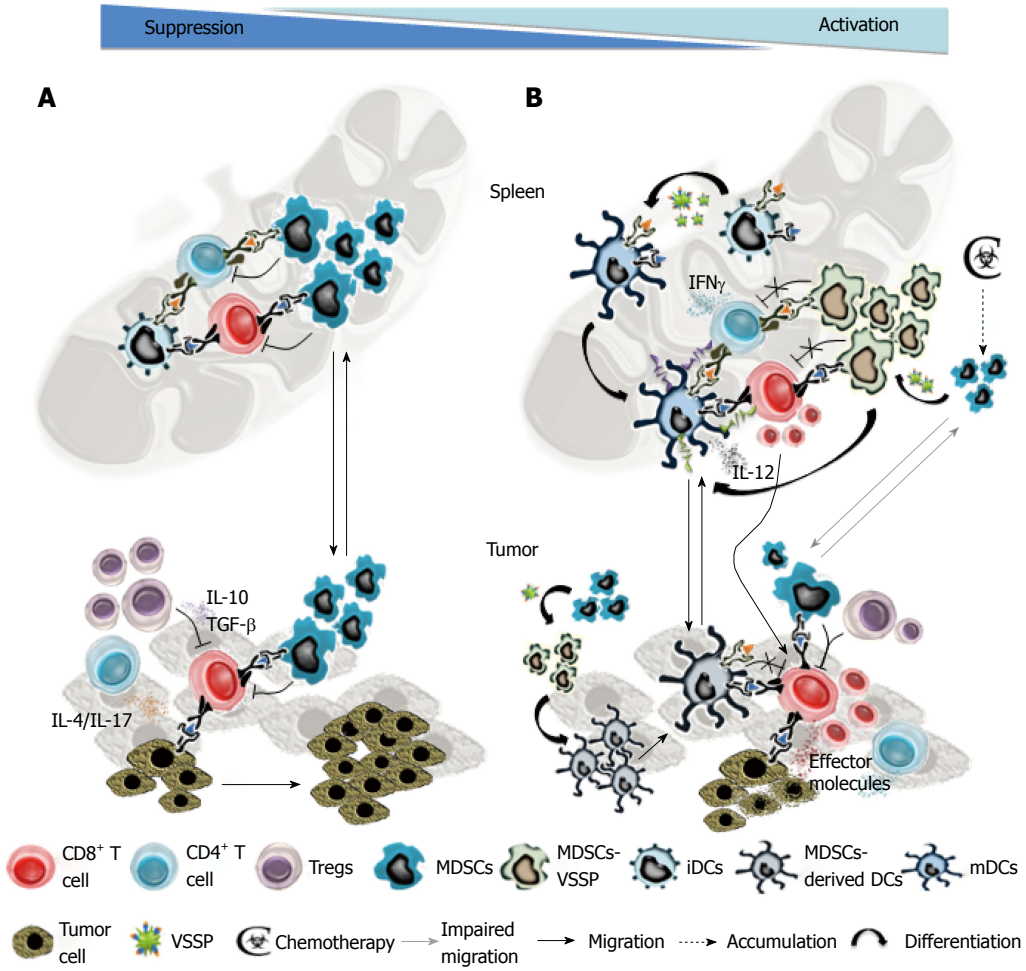

Figure 1 Schematic of potential immunomodulatory effects of very small size proteoliposomes in tumor-bearing hosts. A: Tumor-associated immunosuppressive networks prevent the elimination of neoplastic cells by specific T cells, thus contributing to tumor growth and metastasis; B: VSSP administration reduces the suppressive function of tumor-induced MDSCs, impairs their migration to the tumor microenvironment and promotes their differentiation towards DCs, both at the tumor and secondary lymphoid organs. VSSP also stimulates the activation and effector function of tumor-specific CTL, and combined with the concomitant reduction in the frequency of suppressive MDSCs and Tregs at the tumor site, further enhances elimination of neoplastic cells. An accelerated recovery from chemotherapy-induced leukopenia with VSSP treatment also contributes to a better anti-tumor response. VSSP: Very small size proteoliposomes; MDSCs: Myeloid-derived suppressor cells; DCs: Dendritic cells; CTL: Cytotoxic T lymphocytes; IL: Interleukin; TGF: Transforming growth factor.

ing the modulation of tumor-induced MDSCs by VSSP treatment alone in RCC patients.

CONCLUSION

The immunomodulatory and anti-tumor properties of VSSP are summarized in Figure 1. In tumor-bearing mice, activation and effector function of tumor-specific $CD8^+$ and $CD4^+$ T cells are impaired, among other factors, due to ineffective antigenic presentation by immature DCs and through multiple suppressive mechanisms exerted by MDSCs. Experimental evidence suggest that VSSP-based vaccines could promote cross-presentation of the formulated antigen by DCs, drive the full maturation of the DCs and, simultaneously, inhibit tumor-induced MDSCs immunosuppression. In addition, VSSP could induce Th1 polarization on tumor-specific $CD4^+$ T cells. All these effects may significantly enhance the proliferation and activation of tumor-specific $CD8^+$ T cells, thus eliciting robust anti-tumor immunity. VSSP also diminishes the migration of MDSCs towards the tumor site and promotes their differentiation into DCs. Tumor-infiltrating MDSCs have been related with the recruitment and expansion of Tregs[23,24,73], in addition to an impaired migration of effector T cells[74]. Thus, within the tumor microenvironment, VSSP treatment may tip the the balance between functional T cells vs suppressive MDSCs/Tregs to favor the immune effectors that ultimately lead to an anti-tumor response. The higher frequency of DCs could additionally contribute to activate T cells specific for other tumor antigens by capturing, processing and presenting the proteins released by dying tumor cells. In chemotherapy-treated individuals, VSSP also accelerates the homeostatic recovery of $CD8^+$ T cells and DCs, whereas the suppressive function of chemotherapy-induced MDSCs is abrogated. Altogether, these elements support the use of VSSP as a novel adjuvant or immunomodulator for active immunotherapy and, particularly, for the combination with chemotherapy in the clinical setting.

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