Enhancement of antigen-presenting cell surface molecules involved in cognate interactions by immunostimulatory DNA sequences

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Keywords: activation markers, antigen-presenting cells, CpG motifs, immunostimulatory DNA sequences, Th1 response

Abstract

Bacterial genomic DNA, plasmid DNA (pDNA) and synthetic oligodeoxynucleotides (ODN) containing immunostimulatory DNA sequences (ISS) have been proposed to foster a Th1 response via the release of type 1 cytokines from macrophages, dendritic cells, NK cells and B cells. In this study, we show that ISS-enriched DNA up-regulates a distinct profile of cell surface molecules on macrophages and B cells in vitro and in vivo. ISS-ODN and ISS-containing pDNA enhanced the expression of antigen presentation molecules (MHC class I and II), co-stimulatory molecules (B7-1, B7-2 and CD40), cytokine receptors (IFN-γ receptor and IL-2 receptor), an adhesion molecule (ICAM-1) and an Fc receptor (Fcγ receptor) on murine B cells or bone marrow-derived macrophages. The increased expression of these surface molecules is seen in purified cell populations and is largely independent of the effects of type 1 cytokines. Splenic antigen-presenting cells stimulated with ISS-ODN in vivo efficiently activate naive T cells and bias their differentiation toward a Th1 phenotype in vitro. Thus, the induction of both type 1 cytokines and a distinct profile of cell surface molecules contributes to the potent immunostimulatory effects of ISS-containing DNA on innate and adaptive immunity.

Introduction

The immunostimulatory properties of bacterial DNA were first discovered when it was found that mycobacterial DNA (Bacille Calmette-Guerin) elicited IFN-α, β and γ production and induced NK cell activation (1–3). Subsequent experiments have shown that oligonucleotides containing immunostimulatory DNA sequences (ISS; also known as CpG motifs) induce the release of IL-6, IL-12, tumor necrosis factor (TNF)-α and IL-18, primarily from monocytes and macrophages (4–8). Indeed, recent work has demonstrated the Th1-promoting activity of synthetic oligodeoxynucleotides (ODN) containing ISS sequences (ISS-ODN) on co-administered antigens (9–13). Although the cytokine milieu induced by ISS is thought to play an important role in amplifying the subsequent immune response to the encountered antigen (6,10), cognate interactions between antigen-presenting cells (APC) and lymphocytes or among lymphocytes (e.g., B cell–T cell) also play a crucial role in the primary activation of T and B cells. This study was therefore designed to uncover the relationship between ISS-induced cytokines and the activation of surface molecules on different subsets of immunocytes, and to explore the subsequent functional properties induced by these ISS-driven changes in the induction of the immune response in vitro and in vivo. The results presented here indicate that (i) ISS-ODN induces a distinctive profile of cell surface molecules on APC [B cells and bone marrow-derived macrophages (BMDM)] but not on T cells, (ii) this profile of cell surface markers is only partially dependent on ISS-induced cytokine secretion, and (iii) this distinctive activation profile plays an important role in vivo in the generation of a potent antigen-specific T cell response and its differentiation toward Th1 phenotype.
Methods

Animals
Female BALB/c mice, 6–10 weeks of age, were purchased from The Jackson Laboratory (Bar Harbor, ME). TCR-OVA (DO11.10) transgenic mice on the BALB/c background (14) were generously provided by Dr. Dennis Loh. All experimental animal protocols were approved by the UCSD Animal Subjects Committee.

Nucleic acid reagents and lipopolysaccharide (LPS)
Endotoxin-free (<1 ng/mg DNA) phosphorothioate single-stranded ODN (Trilink, San Diego, CA) were used in all experiments. The sequence of the ISS-ODN is 5′-TGACTGTGAACGTTCGAGATGA-3′ and the sequence of the mutant (M)-ODN is 5′-TGACTGTAAGGTTAGAGATGA-3′ (6). The underline indicates the ISS ( CpG motif) and its corresponding 1 base alteration (CG → GG). The plasmid pUC19 (bacterial plasmid containing ISS) was purified with the Qiagen MaxiPrep kit (Chastworth, CA). Plasmid DNA was phenol–chloroform extracted and ethanol precipitated before use. The endotoxin level was <1 ng/mg DNA or ODN by the Pyrotest limulus amebocyte lysate (Associates Cape Cod, Woods Hole, MA). Escherichia coli LPS was purchased from Sigma (St Louis, MO).

Flow cytometry
Following incubation with Fc block (PharMingen, San Diego, CA), sample cells were stained with conjugated antibodies specific for cell populations and with antibodies specific for surface molecules, as shown in Table 1. Isotype controls for the specific surface markers are as follows: hamster IgG, hamster IgM, rat IgG2a, rat IgG2b, rat IgM, mouse IgG2a and mouse IgG2b (Caltag, San Francisco, CA or PharMingen). Propidium iodide was included in the last wash at a concentration of 2 µg/ml. Live cells (propidium iodide-negative) were analyzed on a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA). The data were analyzed with CellQuest (Becton Dickinson) and FlowJo (Tree Star, San Carlos, CA) software. In most cases, ISS-ODN and LPS treatment significantly increase non-specific antibody binding or autoflorescence (seen as increases in isotype control antibody-stained mean florescence), so this is controlled for with the mean florescence index ratio (MFIR).

Cytokine ELISA and neutralization
The concentrations of cytokines in supernatants were analyzed by commercial ELISA kits (PharMingen or Biosource, Camarillo, CA) according to the manufacturer’s instructions.

For neutralization, murine spleen cells (2×10^6/ml) were cultured in the presence of ISS-ODN (1 µg/ml) plus commercial neutralizing antibodies to IFN-α/β (polyclonal, cat. no. AM42014, 3 µg/ml = 1000 neutralizing units (NU)/ml), IFN-γ (polyclonal, cat. no. AM4034, 10 µg/ml = 1000 NU/ml), TNF-α (polyclonal, cat. no. AM3012, 1 µg/ml = 1100 NU/ml), IL-6 (clone MP5-20F3, 1.25 µg/ml) or IL-12 (clone C15.6, 10 µg/ml) (all from Biosource), or a cocktail of all the above. The concentrations of the various antibodies were pre-determined to neutralize cytokine levels induced by ISS-ODN. The cells were harvested after 48 h incubation and activation markers on B cells (gated on B220^+ population) were analyzed by FACS. As controls, sheep, rabbit (Irvine Scientific, Santa Ana, CA), rat (PharMingen) and hamster (Caltag) sera were used.

Inhibition of B cell proliferation by irradiation or mitomycin C (MMC) treatment
To evaluate the effect of the mitogenic properties of ISS (15,16) on cell surface molecules, spleen cells were γ irradiated (1500 rad) or treated with MMC (50 µg/ml for 30 min at 37°C) prior to stimulation. Cells were then incubated with ISS-ODN (1 µg/ml), M-ODN (1 µg/ml), LPS (5 µg/ml) or media alone for 48 h, then stained for expression of cell surface markers on B cells (Table 1). BrdU staining (17) confirmed inhibition of B cell proliferation by pre-treatment.

Preparation and activation of BMDM
Bone marrow cells were flushed from the femurs of 4- to 8-week-old female BALB/c mice and cultured in 145 mm sterile Petri dishes as previously described (18,19) Primary culture media was DMEM (Irvine Scientific) supplemented with 10% FBS (Gemini Bioproducts), 2 mM L-glutamine (BioWhitaker), 1 mM sodium pyruvate (BioWhitaker), 2 mM penicillin/streptomycin (BioWhitaker) with 30% L cell conditioned media (supernant from L929 cells) (19). The cells had acquired macrophage-like morphology (BMDM), and FACS analysis at day 8 showed the cell surface phenotype of the population to be: >90% Mac3+, <0.2% B220−, >0.4% CD4+ and CD8+ cells. BMDM were washed and re-cultured with supplemented DMEM media or DMEM media containing ISS-ODN (1 µg/ml), M-ODN (1 µg/ml) or LPS (5 µg/ml) for 48 h and then harvested for FACS analysis.

T cell proliferation assay and cytokine analysis
Splenic T cells and APC were prepared from TCR-OVA transgenic mice. To obtain APC, mice were injected i.d. with saline, ISS-ODN (50 µg/mouse) or M-ODN (50 µg/mouse) on days 0 and 7, and sacrificed on day 14. To enrich APC, splenocytes were treated with anti-CD8 antibody (3.155) and anti-CD4 antibody (RL172) followed by incubation with guinea pig complement as described (20), and treated with MMC (50 µg/ml, 30

FlowJo (Tree Star, San Carlos, CA), sample cells were stained with conjugated antibodies specific for cell populations and with antibodies specific for surface molecules, as shown in Table 1. Isotype controls for the specific surface markers are as follows: hamster IgG, hamster IgM, rat IgG2a, rat IgG2b, rat IgM, mouse IgG2a and mouse IgG2b (Caltag, San Francisco, CA or PharMingen). Propidium iodide was included in the last wash at a concentration of 2 µg/ml. Live cells (propidium iodide-negative) were analyzed on a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA). The data were analyzed with CellQuest (Becton Dickinson) and FlowJo (Tree Star, San Carlos, CA) software. In most cases, ISS-ODN and LPS treatment significantly increase non-specific antibody binding or autoflorescence (seen as increases in isotype control antibody-stained mean florescence), so this is controlled for with the mean florescence index ratio (MFIR). MFIR = ([mean florescence when stained for surface molecule] / [mean florescence when stained with isotype control antibody]). MFIR represents the fold increase in surface marker expression relative to background autoflorescence and non-specific antibody binding. We have found MFIR to be a conservative and more accurate estimate of expression of surface molecules when studying cells treated with ISS-containing DNA.

Spleen cell culture and B cell proliferation
Murine spleen cells (2×10^6/ml) were cultured in RPMI supplemented with 10% FBS (Gemini Bioproducts, Calabasas, CA), 2 mM L-glutamine (BioWhitaker, Walkersville, MD), 2 mM penicillin/streptomycin (BioWhitaker) and 50 µM 2-mercaptoethanol (Sigma). Splenic B cells were purified by depletion of non-B cells with antibodies and complement. Briefly, spleen cells were incubated in supplemented RPMI for 1 h (adherent step) and then incubated with anti-CD8 antibody (3.155), anti-CD4 antibody (RL172) and anti-Thy-1 antibody (YTS 154) followed by incubation with guinea pig complement as described (20), and treated with MMC (50 µg/ml, 30

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activation of APC by ISS

Table 1. Antibodies for FACS Analysis in This Study

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<td>macrophages</td>
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*Phycoerythrin.

Results

ISS-ODN induces in vitro up-regulation of a distinctive profile of cell surface markers on B cells from spleen and blood

Incubation for 48 h with ISS-ODN produces a distinct profile of surface molecules on purified splenic B cells (Fig. 1). The expression of MHC class II, CD40 and CD16/32 was clearly up-regulated, and the expression of MHC class I, IFN-γ receptor and IL-2 receptor was slightly up-regulated in MFIR. Interestingly, ISS-ODN suppressed CD23 (Fce receptor) expression. No differences in the expression of CD49b, CD49a, CD49c, Il-1 receptor and IL-6 receptor (data not shown) were observed in the ISS-ODN versus media or M-ODN treated cells. Stimulation with LPS resulted in a similar pattern to that observed for ISS-ODN but with different intensity, except for CD23.

Splenical B cells are considered to have more of an activated/memory phenotype than peripheral cells, so to evaluate whether ISS-ODN could also activate resting naive B cells, lymphocytes were Ficoll purified from mouse peripheral blood. The lymphocytes were cultured in media alone, with ISS-ODN or with M-ODN for 48 h, under the same conditions described above. FACS analysis showed that ISS-ODN suppresses CD49b expression. No differences in the expression of CD49b, CD49c, Il-1 receptor and IL-6 receptor (data not shown) were observed in the ISS-ODN versus media or M-ODN treated cells. Stimulation with LPS resulted in a similar pattern to that observed for ISS-ODN but with different intensity, except for CD23.

While purified B cells alone readily respond to ISS-ODN with surface marker expression, it may be that the complex cellular interactions with other cell types (e.g. macrophages, NK cells) have some enhancing or modulatory effects. To address this, whole splenocytes (after red blood cell lysis) were treated as above with ISS-ODN or controls, and B220<sup>+</sup> cells were analyzed for the full set of surface marker expression profiles. The B cells in whole splenocyte mixtures showed essentially the same profile of cell surface molecule expression as seen in Fig. 1 with purified B cells (data not shown).

min at 37°C) prior to being used as accessory cells. Naive T cells were purified from splenocytes obtained from naive TCR-OVA transgenic mice by depletion with antibody cocktail; J11D (anti-HSA), CA4.12 (anti-Ia), RA36B2 (anti-B220), M5.114 (anti-Ia, isotype IgG2a), MAR.18 (mouse anti-rat IgG) and guinea pig complement. The purity of resultant T cell preparations was >95% by FACS staining. Naive T cells were incubated with the same number of different accessory cells (APC) in the presence of hen egg ovalbumin (OVA, 10–30 µg/ml, grade 5; Sigma). After 4 days incubation, cells were pulsed with <sup>3</sup>H-thymidine (1 µCi/well; ICN Pharmaceuticals, Irvine, CA) for 18 h, then harvested and <sup>3</sup>H-thymidine incorporation was determined with a 1450 Microbeta liquid scintillation counter (Wallac, Turku, Finland). Supernatants were harvested for cytokine assay. Cytokine levels (IFN-γ, IL-4 and IL-5) in supernatants were analyzed by commercial ELISA kits (PharMingen or Biosource).
ISS-ODN induces in vivo up-regulation of cell surface markers on splenic B cells

In order to examine the effect of ISS-ODN to the activation profile in vivo, we injected BALB/c mice with ISS-ODN, M-ODN (100 µg in 200 µl saline per mouse) or saline i.p. Mice were sacrificed at either day 2, 7 or 21 after the injection, splenocytes were stained and surface markers on B cells were analyzed by FACS. Figure 2 shows the activation profile in vivo. The expressions of MHC class I, MHC class II, B7-1, B7-2, CD40, ICAM-1, CD16/32 and IL-2 receptor were slightly up-regulated, and most of them reached their peaks at day 7 except for MHC class I, CD40 and CD16/32. Down-regulation of CD23 expression was observed in vivo, as it was in vitro (Fig. 1).

ISS-induced cytokines only partially mediate ISS-induced cell surface marker up-regulation

In order to evaluate the effects of Th1-type cytokines on the activation markers induced by ISS-ODN, various cytokines (IL-12, IL-6, IFN-α/β, IFN-γ and TNF-α) were neutralized with antibodies in vitro. The levels of these cytokines were initially

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**Fig. 1.** In vitro induction of cell surface molecules on purified mouse B cells. Purified B cells (2×10⁶/ml) obtained from spleens of naive BALB/c mice were cultured with ISS-ODN (1 µg/ml), M-ODN (1 µg/ml) or media alone for 48 h and then stained as described. The x-axis represents a log scale of fluorescence intensity of each surface marker and the y-axis represents the number of B220⁺ cells found of that fluorescence. For each histogram, the surface molecule-specific antibody (black line) is compared to the appropriate antibody isotype control (gray line). In most cases, ISS-ODN and LPS treatment significantly increase non-specific antibody binding (seen with increases in isotype control binding), so this is controlled for with the MFIR, given in the top right corner of each histogram. Additionally, stimulation of B cells with the ISS-containing plasmid pUC19 (at 10 µg/ml) gives the same profile of surface marker expression induction as seen in this figure with ISS-ODN stimulation (data not shown). The induction of surface molecules by ISS-ODN is not dependent upon proliferation because ISS-ODN-stimulated B cells previously treated with γ irradiation or MMC give essentially identical surface marker expression changes as those seen in this figure (data not shown). Data represents results from three independent experiments.

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**Fig. 2.** In vivo induction of cell surface molecules on mouse splenic B cells. BALB/c mice were injected i.p. with ISS-ODN (100 µg), M-ODN (100 µg) or saline. Mice were sacrificed on day 2, 7 or 21 after injection, the spleens were harvested and the spleen cells were stained for FACS analysis. The histograms show the expression of each surface molecule within the B220⁺ cell population, in the same format as that in Fig. 1. Data are representative of the results obtained from three different mice per group.
assessed and the matching concentration of antibody for each cytokine was determined by titration assay until complete neutralization was observed (data not shown). Percent inhibition of activation markers was defined on splenocytes gated on the B220<sup>+</sup> cell population. As shown in Fig. 3, the enhanced expression of MHC class II was not modified by neutralization of cytokines described above. The neutralization of IFN-γ inhibited CD40 and IL-2 receptor expression up to 40%, and had a modest effect on ICAM-1. A cocktail of neutralizing antibodies against all cytokines resulted in 30% inhibition of CD40 and 70% inhibition of IL-2 receptor induction.

**Fig. 3.** Neutralization of cytokines produced in response to ISS partially inhibits the expression of some cell surface molecules. Spleen cells (2 x 10⁶/ml) were cultured in the presence of ISS-ODN (1 µg/ml) and neutralizing antibodies against IFN-αβ, IFN-γ, TNF-α, IL-6 and/or IL-12. The neutralization effect of these cytokines was confirmed by cytokine ELISA, then cells were stained to see the inhibitory effect of cytokine neutralization on activation markers. Percent inhibition of activation markers on the B220<sup>+</sup> cell population was defined as follows: 100% x (MF<sub>ISS</sub> – MF<sub>ISS + neutralizing antibody</sub>) / (MF<sub>ISS</sub> – MF<sub>media control</sub>), where MF is mean florescence. Data represent results from one representative experiment out of three performed.

**Induction of cell surface molecules on B cells is not dependent upon B cell proliferation**

To evaluate whether the effect of ISS-ODN on the expression of surface molecules on B cells was related to its mitogenic effect on B cells (15,16), splenocytes were γ irradiated or treated with MMC and then incubated with ISS-ODN or M-ODN. The expression of surface markers was analyzed 48 h later. The inhibition of proliferation was confirmed by the lack of BrdU incorporation (data not shown). ISS-ODN upregulated the expression of cell surface molecules despite the complete inhibition of B cell proliferation either by irradiation or by MMC treatment (data not shown), indicating that the up-regulation of surface molecules by ISS-ODN is not due to its of mitogenic effect.

**ISS-ODN does not induce an activation of cell surface molecules on splenic T cells**

We also evaluated the differential expression of antigen presentation and activation cell surface molecules on T cells such as CD28, CTLA-4 and CD40 ligand in addition to the markers described for B cells (Figs 1 and 2). Incubation of splenocytes with ISS-ODN did not modify any of these cell surface molecules on splenocytes gated on the CD4<sup>+</sup> or CD8<sup>+</sup> cell population in vitro (data not shown). In addition, injection with ISS-ODN in vivo also did not modify cell surface molecules on splenocytes gated on the CD4<sup>+</sup> or CD8<sup>+</sup> cell population (data not shown).

**ISS-ODN induces up-regulation of a distinctive profile of cell surface markers on BMDM in vitro**

Recent work has demonstrated the role of ISS-ODN in activation of macrophages to release various cytokines such as TNF-α, IFN-αβ and IL-12 (4–8). In this study we analyzed the effect of ISS on the pattern of cell surface molecules expressed on macrophages. As shown in Fig. 4, ISS-ODN clearly

![Fig. 4. Induction of cell surface molecules on BMDM. BMDM (2 x 10⁶/ml) were incubated in vitro with ISS-ODN (1 µg/ml), M-ODN (1 µg/ml), LPS (5 µg/ml) or media alone for 48 h, and then stained for Mac-3 (phycoerythrin) and activation markers (FITC) for FACS analysis. The histograms represent the FITC staining of gated Mac-3<sup>+</sup> macrophages, in the same format as that in Fig. 1. Additionally, stimulation of BMDM with the ISS-containing plasmid pUC19 (at 10 µg/ml) gives the same profile of surface marker expression induction as seen in this figure with ISS-ODN stimulation (data not shown). Data represents results from one representative experiment out of three that were performed.](image-url)
enhances the expression of MHC class I, B7-1, CD40, ICAM-1 and CD16/32 on BMDM.

pUC19 induces up-regulation of surface molecules on B cells and BMDM

In previous work we and others have proposed that in gene vaccination, the pDNA serves not only to encode the antigen, but also as its intrinsic adjuvant (9,10,21). This effect is mediated via the ISS motifs in the plasmid backbone (6,9,21). To analyze the effect of pDNA on the cell surface profile, we either incubated or transfected mouse splenocytes with pUC19 or methylated pUC19 for 48 h prior to analysis by FACS. Indeed, pUC19 but not methylated pUC19 induced a similar cell surface expression profile on B splenocytes as well as on BMDM under each of these conditions (data not shown) as observed for ISS-ODN (see Figs 1 and 4 respectively).

ISS-ODN enhanced the functionality of splenic APC to activate and to induce the differentiation of naive T helper cells

The data presented above indicate that ISS-ODN up-regulates a distinct profile of cell surface molecules in addition to promoting cytokine production (4–8). To evaluate the functionality of this expression profile, splenic APC were obtained from TCR-OVA transgenic mice injected with ISS-ODN, M-ODN or saline and their ability to activate naive T cells in the presence of OVA in vitro was examined. Indeed, splenic APC obtained from mice injected with ISS-ODN induced 4-fold more T cell proliferation (at 30 μg/ml of OVA) than splenic APC obtained from mice injected with saline or M-ODN (Fig. 5A). This indicates that treatment with ISS-ODN enhanced the capacity of the APC to stimulate naive T cells. Furthermore, the subsequent activation of naive T cells resulted in OVA-specific IFN-γ production (Fig. 5B), indicating the differentiation of naive T<sub>H</sub> cells toward a T<sub>H</sub>1 phenotype by APC treated with ISS-ODN.

Discussion

ISS-ODN has been shown to activate NK cells and macrophages to secrete a profile of cytokines including TNF-α, IFN (α, β and γ), IL-12 and IL-18 (2–8). While these cytokines are thought to play major roles in the amplification of the immune response toward the encountered antigen (6,11–13), this study indicates that the adjuvanticity of ISS-ODN is also mediated via the induction of a distinctive profile of cell surface molecules involved in cognate interaction.

ISS-ODN enhanced the expression of several categories of cell surface molecules involved in antigen presentation on splenic and peripheral B cells in vitro and in vivo. Antigen presentation may be directly enhanced by the up-regulation of MHC class I and II. Also, ISS-ODN slightly enhanced the expression of co-stimulatory molecules, such as B7-1, B7-2 and CD40 in vivo. These molecules play a major role in T cell priming, activation and differentiation as well as in T–B cell collaboration. While the role of B7/CD28 interaction in regulating T<sub>H</sub>1 and T<sub>H</sub>2 differentiation is complex and not fully resolved (22–24), there is clear evidence for the role of CD40–CD40 ligand co-stimulation in T<sub>H</sub>1 differentiation, B cell activation and isotype switching (25–28). CD40–CD40 ligand co-stimulation regulates these effects via the up-regulation of

B7 expression on APC, and via the induction of IL-12 from monocytes, macrophages and dendritic cells (29). Thus, the induction of co-stimulatory molecules on APC by ISS-ODN may provide the important signals for T<sub>H</sub>1 differentiation (30).

ISS-ODN increases the expression levels of IL-2 receptor and IFN-γ receptor on B cells, but does not up-regulate the expression of other cytokine receptors such as IL-1 receptor or IL-6 receptor. The selective up-regulation of these cytokine receptors can enhance the in vivo B cell proliferative response, Ig synthesis and isotype switching to IgG2a upon exposure to the corresponding cytokines (6,10).

The up-regulation of cell surface molecules on B cells was observed for both splenic B cells (mainly memory cells) and peripheral blood B cells (mainly naive cells). The levels of expression in vivo were maximal 1 week after i.p. injection of ISS-ODN and dropped back to the baseline levels after 21 days for most of the parameters evaluated, except for MHC class I and CD40 which both displayed sustained expression.
Gamma irradiation or MMC treatment of B cells did not modify the expression of the various cell surface molecules, ruling out the mitogenic effect of ISS on B cells for this differential expression. Cytokine neutralization resulted in a partial inhibition of the differential expression of some surface molecules on B cells, indicating the limited role of type 1 cytokines in the observed expression profile. In response to ISS-ODN in vivo and in vitro, CD4+ and CD8+ cells did not significantly increase their expression of a wide variety of surface molecules, although a recent paper has shown a modest indirect induction of some markers in response to ISS-containing Drosophila genomic DNA (31).

ISS-ODN enhanced the expression of surface molecules on BMDM. The expression of MHC class I, B7-1, CD40, ICAM-1, and CD16/32 was clearly up-regulated on BMDM, while expression levels of MHC class II were not clearly modified. Notably, the up-regulation of ICAM-1 was more pronounced on BMDM than on B cells. ICAM-1 stabilizes interactions between APC and lymphocytes by binding to its ligand LFA-1, thus reducing the threshold number of cognate interactions required for activation (32). Recently, it has been demonstrated that dendritic cells also can be stimulated by ISS-containing DNA to up-regulate a similar profile of surface molecules (33,34).

In recent studies we have proposed that ISS (CpG motifs) in the pDNA backbone induce the necessary initial cytokine milieu (IFN and IL-12) which fosters the characteristic Th1 response seen in gene vaccination (6,9). In light of this finding, the pDNA provides a source for both antigen and adjuvant (10). Indeed, pUC19, but not methylated pUC19, up-regulated the expression levels of cell surface molecules on B cells or BMDM in vitro, with a similar profile to that observed with ISS-ODN stimulation. This suggests that the role of ISS as an adjuvant in the plasmid backbone of DNA vaccines is not limited to cytokine stimulation, but also includes increases in important cell surface molecules.

It has previously been shown that the local cytokine milieu (35,36) and the expression of surface molecules on APC (37) enhance the subsequent immune response. In our study splenic APC stimulated with ISS in vivo enhanced the T cell proliferative response to antigen stimulation and induced OVA-specific IFN-γ production in vitro. This indicates that ISS-activated splenic APC can instruct and bias the subsequent Th1 response toward a Th1 phenotype, emphasizing the instructive role of innate immunity (i.e. APC) in the development of the adaptive immune response (38).

In summary, this study demonstrates that ISS-ODN or pDNA including ISS up-regulates cell surface receptors involved in cognate interactions. These include antigen presentation (MHC class I and II), co-stimulation (B7 and CD40), antigen uptake (Fcγ receptor), cell–cell adhesion (ICAM-1) and cytokine receptors. Combined with cytokine production, i.e. TNF-α, IFN, IL-12 and IL-18, ISS-induced increases in these surface molecules appear to provide the basis for the observed strong Th1-inducing adjuvant properties. ISS-ODN were initially discovered in the mycobacterial genome as DNA sequences that enhanced NK cell activity (2). Indeed, a recent study demonstrated that the infection of human dendritic cells with mycobacteria resulted in up-regulation of MHC class I, B7, CD40 and ICAM-1, and the release of IL-12 and TNF-α (39), an activation profile which is very similar to the pattern induced by ISS in this study. Thus, microbial ISS plays a major role in priming and activation of APC while synthetic ISS-ODN mediate these effects without the risks posed by infection. These immunostimulatory properties of synthetic ISS-ODN could be applied in vaccine design against infectious and malignant diseases as well as for allergen immunotherapy.

Acknowledgements

We wish to thank Arash Ronaghy, Pei-Ming Chen and Patricia Charos for their excellent technical assistance; Dr. Hans Spiegelberg, Dr. Dennis A. Carson, Dr. Maripat Corr and Dr. Malini Sen for helpful discussions and the review of the manuscript; and Nancy Noon and Jane Uhlle for editorial assistance. This work was supported in part by NIH grant Al-40682 and by Dynavax Technologies Corp. E. M.-O. is a recipient of a fellowship from the Spanish Ministry of Education and Culture (MEC); H. K. is a recipient of a scholarship from the Fukushima Medical Foundation. J. V. U. is a Medical Scientist Training Program Trainee (NIGMS grant GM 07198) and a Lucille P. Markey Charitable Trust Fellow.

Abbreviations

- APC: antigen-presenting cell
- BMDM: bone marrow-derived-macrophages
- ISS: immunostimulatory DNA sequences
- ISS-pDNA: pDNA containing ISS
- ISS-ODN: ODN containing ISS sequences
- LPS: lipopolysaccharide
- MFIR: mean florescence index ratio
- MMC: mitomycin C
- M-ODN: oligodeoxynucleotides containing the mutant sequence
- NU: neutralizing units
- ODN: oligodeoxynucleotide
- OVA: ovalbumin
- pDNA: plasmid DNA
- TNF: tumor necrosis factor

References


