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Review

Prophylactic vaccines mimic synthetic CpG oligonucleotides in their ability to modulate immune responses

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ABSTRACT

Synthetic oligonucleotide ligands that bind to toll-like receptors are known to modulate the immune response via the activation of antigen presenting cells, and were therefore proposed as a novel form of vaccine adjuvant. Clinical-grade they are, however, not readily available. Here, we show that commonly used prophylactic vaccines for infectious diseases like measles, mumps and tuberculosis exhibit the same immune modulating behavior as synthetic CpG oligonucleotides in terms of their ability to stimulate IFN-α production and plasmacytoid dendritic cell maturation. Featuring the additional advantages of low-cost and proven safety, these vaccines could therefore be attractive alternatives to CpG oligonucleotides as adjuvants for immunotherapy. This previously undiscovered characteristic of prophylactic vaccines also sheds new light on the mechanisms by which they operate and is extremely interesting for vaccine development. Moreover, the finding that prophylactic vaccines trigger TLRs like synthetic oligonucleotides opens the possibility to predict the immune response of new vaccines.

1. Introduction

Tremendous progress has been made in the fight against infectious diseases through the use of vaccines. Despite their efficacy, the mechanism by which these universally applied vaccines potentiate the immune system has not been fully elucidated (Querec et al., 2006). Dendritic cells (DCs) are among the first immune cells to encounter vaccines through their pathogen-recognition receptors, of which Toll-like receptors (TLRs) are the most prominent subclass (Kaisho and Akira, 2000). Plasmacytoid DCs (pDCs), which link innate with adaptive immune responses by secretion of interferon (IFN) and induction of costimulatory- and antigen-presenting molecules, play a prominent role in the initial process of pathogen recognition (Hemmi and Akira, 2005; Kaisho and Akira, 2003; Montoya et al., 2006). Besides cytokine secretion needed for B- and T-cell activation, TLR-activated DCs present processed antigens to stimulate antigen-specific T-cells, ultimately leading to long lasting pathogen-specific T-cell memory and protective antibody production by B-cells.

Stimulatory effects of bacterial and viral DNA/RNA are ascribed to unmethylated CpG oligonucleotide motifs (Hemmi et al., 2000; Krieg, 2002a; Sugiyama et al., 2005). Based on these motifs, different classes of synthetic CpGs were designed to stimulate the immune system (Krieg, 2002b). These synthetic CpGs mimic the immune-stimulatory effects of bacterial DNA and are recognized through TLRs. Classification of CpGs is based on the immune cell subsets that recognize the CpG motifs via TLR9, each subset corresponding to a different downstream effect (Bhattacharjee and Akira, 2006). In man, the different CpG subclasses are based on their immunological effect on purified B-cells and pDCs – the only cells in the peripheral blood that express substantial levels of TLR9 (Kerkmann et al., 2003). To date, three classes of chemically modified CpGs with different sequence motifs were developed: the A-, B- and C-classes, each having a different immune-stimulatory activity (Hartmann et al., 2003). These chemically designed CpGs are explored for their potential therapeutic effects, f.e., in immunotherapy for cancer, allergies and infectious diseases (Barchet et al., 2008; Corey, 2007).

Somewhat surprisingly, universally applied vaccines, which are known to contain RNA and DNA motifs, have not previously been explored in respect to their capacity to direct immune responses through TLR9. In this study, we identified vaccines used in everyday clinical practice that exhibit remarkably similar behavior to chemically synthesized CpG classes in being able to skew immune response towards either the innate or the adaptive immune system. Our results unfold a new application (Schreibelt et al., 2010) for these clinically approved and widely used therapeutic vaccines. The fact that these vaccines are also cheap, safe and readily avail-

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Table 1
Listed are the 15 vaccine formulations tested for their capacity to stimulate pDCs. Freshly isolated pDCs need a signal like IL-3 to survive in vitro. Eight vaccines (Prevenar, Tetanus, NEISVAC-C, HAVRIX, HbVaxPro, Infanrix, Pneumo and Influvac) appeared not to provide this survival signal and the cells died within 16 h. These vaccines were excluded from further testing. Seven vaccines were able to provide a survival signal to the pDCs, of which five vaccines were stimulatory either measured by enhanced cytokine production or by the upregulation of CD80 and cell-surface major histocompatibility complexes (Fig. 1A and B). To further enhance antibody responses, in some instances adjuvants are added to vaccine preparations. To exclude the possibility that these vaccine adjuvants play a major role in the observed pDC activation, vaccine preparations that share the same adjuvant were compared. Since neither all the vaccines containing aluminium hydroxide nor all the vaccines containing aluminium phosphate induced pDC activation, a prominent role for the adjuvants per se can be excluded.

Infectious agent	Vaccine	Disease	Type of vaccine	Adjuvant
Bacteria				
S. pneumoniae	PREVENAR	Pneumonia, meningitis	Conjugated subunit	$AlPO_4$
S. pneumoniae	PNEUMO 23	Pneumonia, meningitis	Subunit	None
C. tetani	Tetanus	Tetanus	Subunit	$AlPO_4$, thiomersal
S. typhi	TYPHIM Vi	Typhoid fever	Subunit	None
H. influenza type B	Act Hib	Meningitis, Pneumonia type b	Conjugated subunit	Tetanus toxoid
N. meningitidis	NEISVAC-C	Meningitis, sepsis	Conjugated subunit	$Al(OH)_3$, tetanus toxoid
M. bovis	BCG	Tuberculosis	Live attenuated	None
Viruses				
Hepatitis A	HAVRIX	Liver disease, cancer	Inactivated	AlO(OH)
Hepatitis B	HBVAXPRO	Liver disease, cancer	Recombinant subunit	$AlPO_4$
Tick borne encephalitis virus	FSME	Tick borne encephalitis	Inactivated	$Al(OH)_3$
Rabies virus	Rabies	Rabies	Inactivated	Neomycin
Yellow fever virus	STAMARIL	Jaundice, kidney and liver failure	Live attenuated	None
Measles virus Mumps virus Rubella virus	MMR	German measles, respiratory tract infection, mumps, Meningitis, orchitis	Live attenuated	None
C. diphteriae		Difteria	Subunit, inactivated, conjugated	$AlPO_4$, AlO(OH), tetanus toxoid
C. tetani		Tetanus		
B. pertussis		Pertussis		
Poliovirus		Poliomyelitis, paralysis		
H. influenza type B		Meningitis, epiglottitis, pneumonia type b		
Influenza virus A	INFLUVAC 2006–2007	Flu, respiratory disease	Inactivated subunit	None
Influenza virus B	INFLUVAC 2007–2008	Flu, respiratory disease	Inactivated subunit	None

able strongly supports their use as adjuvants, providing clinicians with a valid alternative to the currently expensive and difficult to obtain clinical grade synthetic TLR ligands.

2. Materials and methods

2.1. Cells and reagents

Cells were obtained from healthy volunteers according to institutional guidelines. PDCs were purified using anti-BDCA-4-conjugated magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) and adjusted to 10^6 cells/ml in X-VIVO-15 (Lonza, Basel, Switzerland) in 5% human serum, supplemented with 10 ng/ml IL-3 (Cellgenix, Freiburg, Germany), 5 μg/ml CpG-A (ODN 2216), or CpG-B (ODN 2006), or CpG-C (M362) (Alexis Biochemicals, Lausen, Switzerland) or vaccines (1:10) listed in Table 1.

2.2. Phenotype

Flowcytometric phenotyping was performed using monoclonal antibodies and isotype controls: anti-HLA-ABC (W6/32), anti-HLA-DR/DP (Q5/13) and anti-CD80 (Becton&Dickinson, Mountain View, CA, USA); anti-CD83 (Beckman Coulter, Mijdrecht, Netherlands), anti-CD86 (Pharmingen, San Diego, CA, USA), followed by goat-anti-mouse PE.

2.3. Chloroquine, TLR9 antagonist, single stranded, PMXB

Cells received 50 μM Chloroquine 30 min before stimulation with CpG-A, CpG-C or the vaccines. The TLR9 antagonist TTAGGG (Invivogen, San Diego, CA, USA) was used for inhibition of CpG-A and CpG-C responses. Single-stranded CpG-A and the vaccine MMR were prepared by heating at 95 °C for 5 min followed by flash cooling in dry ice for 10 min. CpG-B and the vaccines Act-Hib and BCG were mixed with polymyxin B (PMXB, Sigma–Aldrich; 100 μg/ml) and allowed to aggregate for 30 min at room temperature.

2.4. Cytokine detection

Supernatants were collected from stimulated pDCs after 16 h culturing. IFN-α was analyzed using standard ELISA procedures (BenderMed Systems, Vienna, Austria).

2.5. Cellular responses

Allogeneic T cells were co-cultured with CpG-C- or FSME-matured pDCs (pDC:T-cell ratio 1:20 with 1×10^5 peripheral blood lymphocytes). After 4 days of culture, 1 μCi/well of tritiated thymidine was added for 16 h and incorporation was measured.

T-cell responses against keyhole limpet hemocyanin (KLH) were measured in a proliferation assay. KLH-specific T-cells were isolated from blood of patients previously vaccinated with KLH-loaded monocyte-derived DC. Lymphocytes were cultured with autologous pDCs with or without KLH and matured with CpG-C or FSME. After 4 days of culture, 1 μCi/well of tritiated thymidine was added for 16 h at day four and incorporation was measured.

2.6. Statistics

All cultures were performed in triplicate and results are shown as the mean ± SD. Significant difference from control according to Student's *t* test.

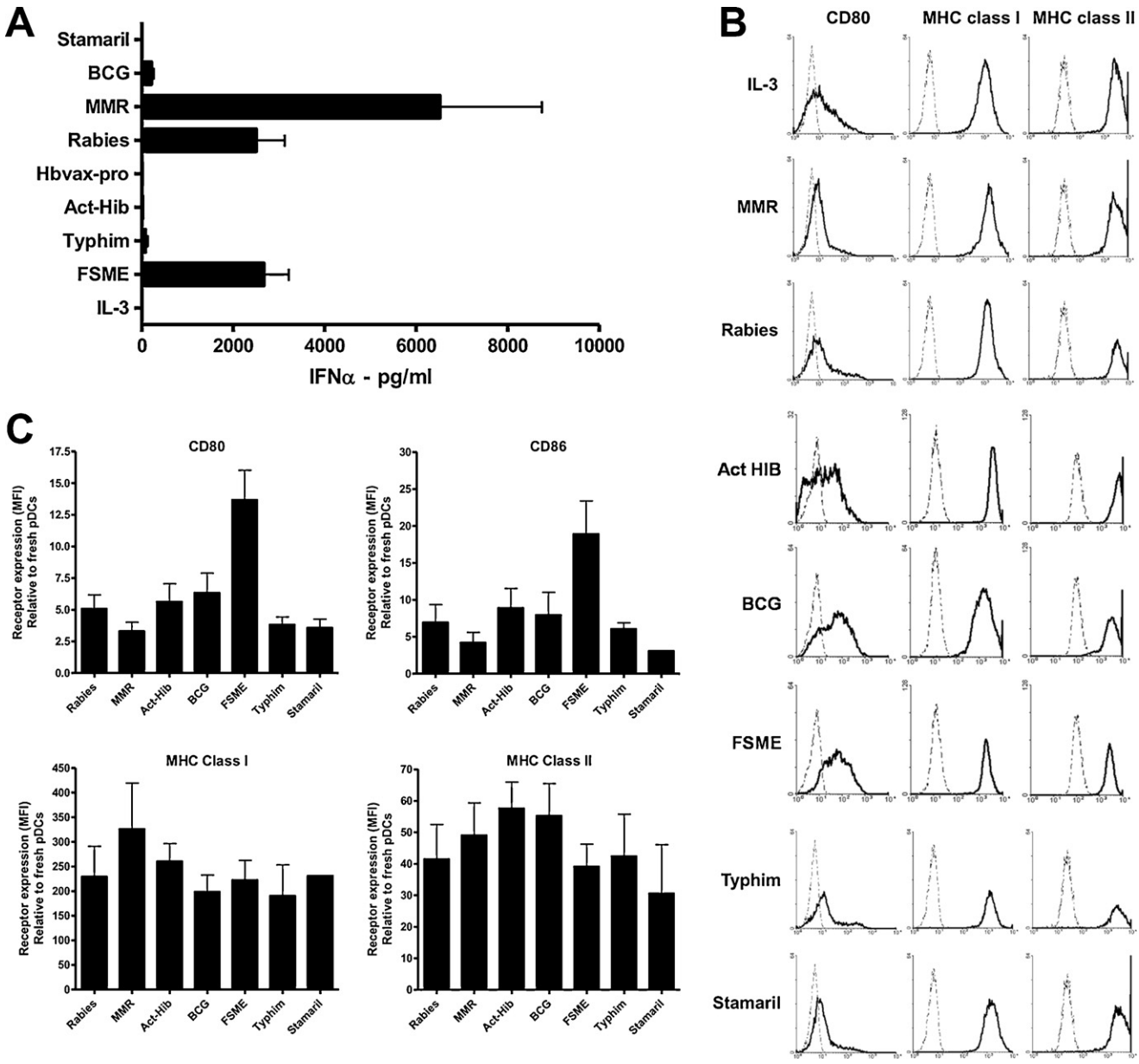

Fig. 1. Stimulation of pDC by vaccines. pDCs were cultured with indicated vaccines (10% v/v) for 16 h. (A) Supernatants were harvested and IFN-α production was evaluated. Averages of three independent donors are shown. (B) Cells were stained with mAbs against CD80, MHC class I and MHC class II. A representative experiment is shown ($n = 6$). Increase in mean fluorescence intensity induced by the indicated vaccines, (bold line) compared to fresh pDCs (thin line) is shown. (C) Vaccines were tested for their capacity to provide a maturation signal. Cells were stained with mAb against CD80, MHC class I and MHC class II. Expression of CD80 and MHC molecules was compared to expression of those markers on freshly isolated pDCs. One representative experiment out of three is shown.

3. Results

3.1. Prophylactic vaccines activate pDCs in distinct manners

Fifteen vaccines were tested for their immune modulating capacity on pDCs (Kadowaki et al., 2001). As freshly isolated pDCs need a signal to survive in vitro this characteristic was tested as well. In man pDCs have a restricted TLR expression profile (TLR7 and TLR9) and, because of their secretion of high levels of IFN-α, have the ability to link innate and adaptive immunity. Seven vaccines sustain pDC survival in vitro similarly to the cytokine IL-3, a pDC survival factor. In addition to prolonging survival, FSME, MMR and Rabies vaccine induced IFN-α production (Fig. 1A). FSME, Act-Hib and BCG upregulated MHC class I and II molecules, which are important for antigen presentation on pDCs along prolonging pDC survival (Fig. 1B). FSME and to a lesser extent Act-Hib and BCG increased the expression of co-stimulatory molecules such as CD80, CD86 and the DC maturation marker CD83 (Fig. 1B). This latter mature phenotype is of significance since non-activated pDCs are immunosuppressive rather than immune stimulatory (Goubier et al., 2008). MMR and Rabies vaccines exhibited no maturation effect (Fig. 1B). Two other vaccines, Stamaril and Typhim, neither induced IFN-α secretion nor phenotypical maturation (Fig. 1A and B).

These vaccines behaved remarkably similar as IL-3 stimulation, resulting in pDC survival without IFN-α secretion or phenotypical maturation. Compared to freshly isolated pDCs upregulation of costimulatory molecules for some of the vaccines is evident (Fig. 1C). So, we concluded that selected vaccines effectively stimulate pDCs and simultaneously, these vaccines modulate pDC phenotype and

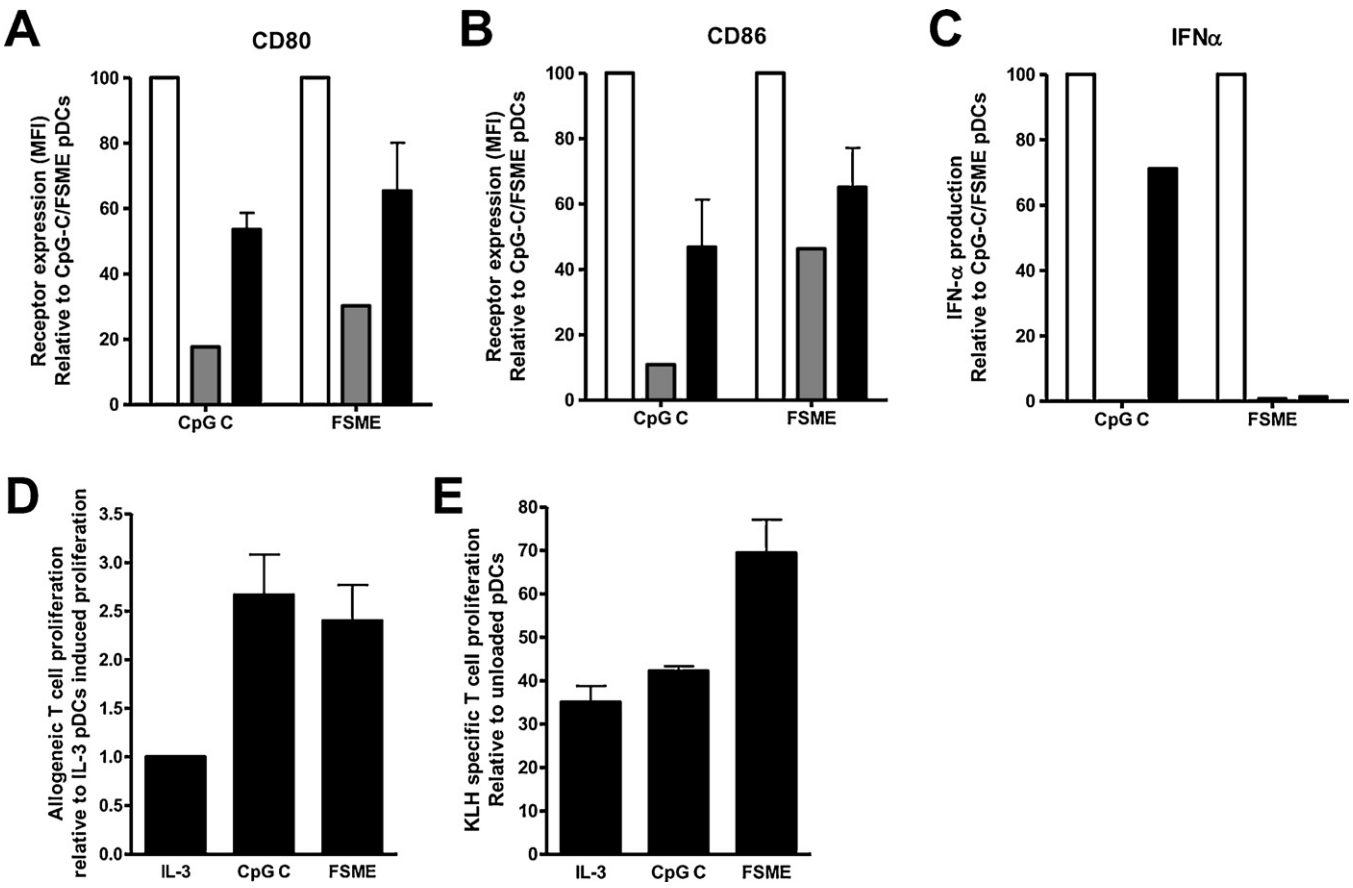

Fig. 2. Vaccines exert their effect on pDC via TLR9 similar to chemically synthesized CpGs based on chloroquine treatment. (A–C) Purified pDCs were activated with CpG-C or FSME either alone or in the presence of chloroquine or a TLR9 antagonist. Relative surface expression of CD80 (A) and CD86 (B) and IFN-α (C) production are depicted. Activation of pDCs by FSME/CpG-C (white bars) induced expression of both CD80 (A) and CD86 (B). In the presence of chloroquine (grey bars) or TLR-9 antagonist TTAGGG (black bars) the upregulation of these molecules was diminished. Likewise, the production of IFN-α (C) induced by stimulation with CpG-C or FSME was largely inhibited by chloroquine treatment and the TLR9 antagonist. (D and E) Purified pDCs activated with either CpG-C or FSME is able to induce T cell proliferation measured by tritiated thymidine incorporation in cpm. (D) Allogeneic T cell proliferation induced by differently activated pDCs depicted as stimulation index. (E) PDCs were purified with BDCA-4 beads from patient material previously vaccinated with KLH-loaded monocyte-derived DCs (de Vries et al., 2003) and cocultured with autologous KLH-responsive PBLs. Due to the presence of antibodies, pDCs are able to take up KLH and present it in MHC class II (Benitez-Ribas et al., 2006). KLH specific T cell proliferation depicted as stimulation index calculated over unloaded pDCs.

function. Some vaccine preparations induced high levels of IFN-α (e.g., Rabies) or induced an immunostimulatory DC phenotype (e.g., Act-Hib), whereas others exhibited a combination of these effects (e.g., FSME).

3.2. Prophylactic vaccines activate pDCs in the same manner as CpG oligonucleotides

Surprisingly, the various properties of selected vaccine affecting pDC function and phenotype are remarkably similar to those of synthetically generated TLR9 targeting CpG compounds and compounds such as imidazoquinolines (e.g., R848), loxoribine and bropirimines synthesized for TLR7 triggering (Jurk et al., 2002; Krieg and Vollmer, 2007). Similar to the Rabies and MMR vaccines, stimulation of pDCs with CpG-A leads exclusively to the production of IFN-α (Fig. 1A), whereas identical to Act-Hib and BCG, stimulation with CpG-B promotes upregulation of antigen presenting MHC molecules (Fig. 1B), supporting adaptive immunity (Kerkmann et al., 2003). Stimulation of pDCs with CpG-C or all other to date described ligands for TLR7 leads to cytokine production and maturation, bringing together the two properties (stimulating antigen presentation and stimulation of adaptive immunity) (Hartmann et al., 2003), a phenomenon we observed for FSME (Fig. 1A and B).

3.3. Prophylactic vaccines activation of pDCs is mediated via TLR

This similarity between synthetic TLR ligands and vaccines prompted us to investigate whether the vaccine induced effects were indeed TLR mediated. Since, chloroquine effectively prevents endosomal maturation through inhibition of vesicular acidification, which has been proven essential prerequisite for CpG-induced signals (Krieg, 2002a), pDCs were stimulated with vaccine preparations in the presence and absence of chloroquine. Treatment of pDCs with chloroquine completely abolished IFN-α secretion and maturation of vaccine-activated pDCs (Fig. 2 A–C), indicating that the effects induced by the selected vaccines are similar to the synthetic TLR ligands and dependent on endosomal maturation. TLR9 specificity is supported by complete elimination of Rabies-, MMR- and FSME-induced IFN-α production by pDCs by TLR9 antagonist TTAGGG (Fig. 2C). Also, simultaneous incubation of the FSME vaccine and the TLR9 antagonist showed no further upregulation of the costimulatory and antigen presenting molecules (Fig. 2A and B). Together, these findings strongly indicate that immunomodulation by these vaccines is mediated through TLR9. No effect of the TLR9 antagonist was observed on Act-Hib/BCG vaccine-induced MHC expression on pDCs. This is in accordance with earlier observations that TLR9 antagonists can inhibit CpG-A and CpG-C but not CpG-B induced effects (Trieu et al., 2006). Indeed, both Act-Hib and BCG show a CpG-B like type of response. CpG-C induces both IFN-α

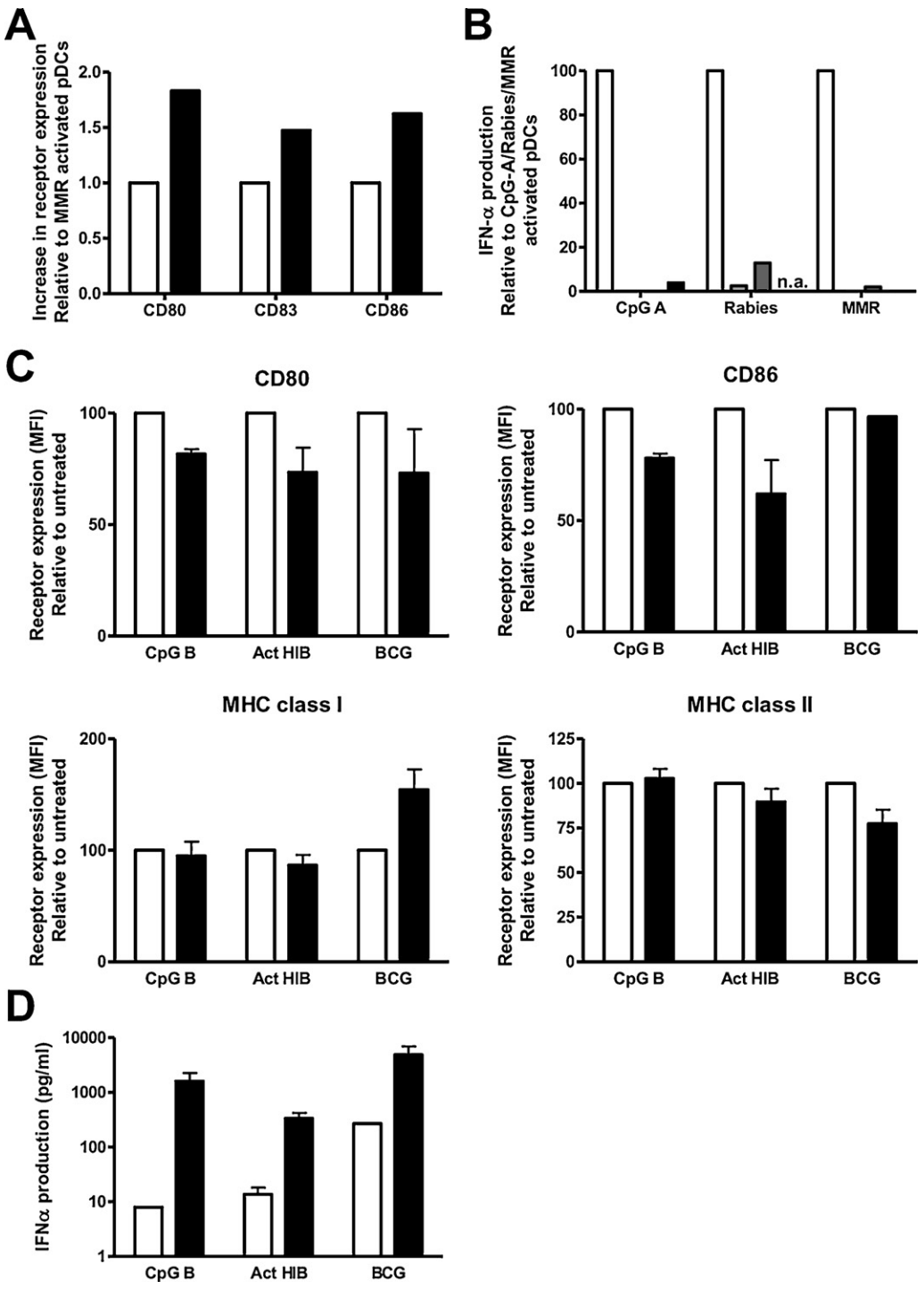

Fig. 3. Vaccines exert their effect on pDC via TLR9 similar to chemically synthesized CpGs based on quarternary state. (A–D). Purified pDCs were activated with the indicated CpGs or vaccines either alone or in the presence of chloroquine and TLR9 antagonist, after the stimuli were made single-stranded or were complexed with PMXB. (A) Expression of costimulatory molecules CD80 and CD86 and DC-specific molecule CD83 was not enhanced by stimulation with the MMR vaccine (white bars). Since the MMR vaccine by itself does not induce phenotypical maturation, the molecule expression levels were set at 1. Upon heating and flash cooling, the MMR vaccine was made single-stranded (black bars) and thereby gained the capacity to induce a mature phenotype characterized by the upregulation of CD80, CD83 and CD86 by pDCs. (B) IFN-a production by pDCs induced by CpG-A or the vaccines Rabies and MMR (white bars) was completely inhibited by chloroquine treatment (light grey bars), TLR9 antagonist (dark grey bars) and after CpG-A and MMR were made single-stranded (black bars). The Rabies vaccine could not be made single stranded because it became semi-solid after heating and flash cooling (n.a., not analyzable). (C and D) CpG-B and the vaccines Act-Hib and BCG (white bars) induced phenotypical activation by upregulation of CD80, CD86, MHC class I and II (C) pDCs were hardly able to induce IFN-a production (D) After complexing with PMXB (black bars) these stimuli lost the capacity to induce full phenotypical activation (C), but gained the capacity to induce high levels of IFN-a production by pDCs (D).

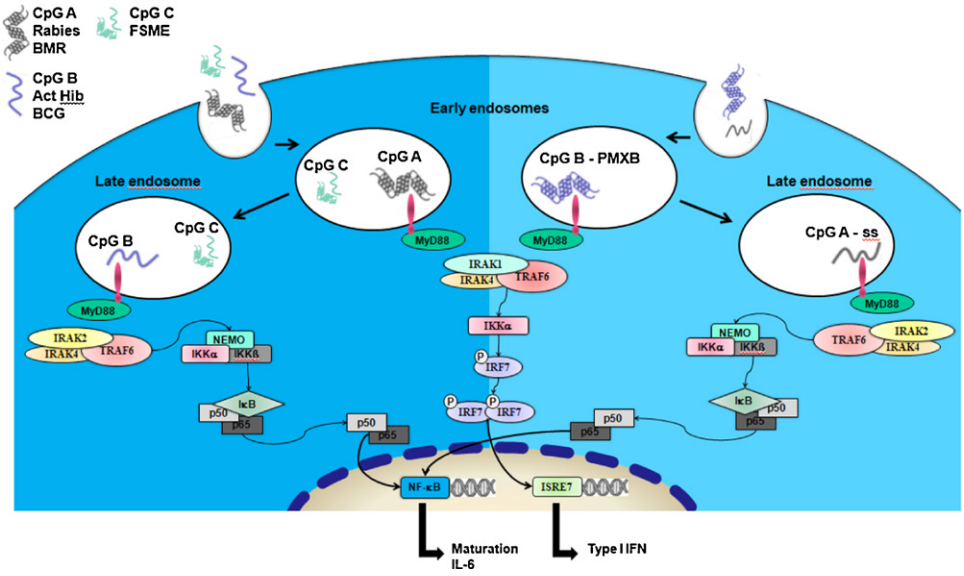

Fig. 4. Signaling pathways exploited by the different prophylactic vaccines and CpG oligonucleotides.

and maturation of pDCs, resulting in a potent antigen presentation to induce T-cell proliferation. FSME-matured pDCs were similar to CpG-C matured pDCs in their capacity to induce allogeneic (Fig. 2D) as well as autologous (Fig. 2E) T-cell responses (Benitez-Ribas et al., 2006,2008). This underscores the similarity between FSME vaccines and synthetic CpG-C.

3.4. Activation of pDCs by vaccines follows distinct CpG subclasses

The activity of Rabies and MMR corresponds with CpG-A, which binds to TLR-9 localized in transferrin receptor positive early endosomes. Induction of IFN-α by CpG-A is likely due to the ability of this subtype to form aggregates via its poly-G tail. Single stranded CpG-A loses this capacity to induce IFN-α production but acquires the competence to induce pDC maturation (Guiducci et al., 2006). To underscore the argument that the effects of Rabies and MMR vaccines on pDCs are identical to those of synthetic CpG-A, Rabies and MMR were rendered single stranded. We demonstrated that single-stranded MMR indeed resulted in a significant decrease in IFN-α production and enhanced phenotypic maturation (Fig. 3A and B). Therefore, the ability of MMR to induce IFN-α directly depends upon multimeric structures, demonstrating the similarity between MMR and synthetic CpG-A.

Along the same lines, Act-Hib and BCG vaccines share the same properties as CpG-B: very low IFN-α production although induction of pDC maturation (Fig. 3C). CpG-B in a monomeric form localizes to LAMP-1-positive late endosomes (Latz et al., 2004). TLR9 ligands present in these endosomal compartments induce pDC differentiation. Complexing CpG-B molecules in large microparticles transforms this component into a potent inducer of IFN-α (Guiducci et al., 2006). To extend the equivalence between CpG-B and the vaccines Act-Hib and BCG, they were mixed with polymyxin B (PMXB), resulting in large structures. Completely in line with CpG-B, this PMXB treatment transformed the Act-Hib and BCG vaccines into potent inducers of IFN-α and eliminated their ability to induce pDC maturation (Fig. 3D). Taken together, the vaccines Act-Hib and BCG are therefore equivalents of synthetic CpG-B.

4. Discussion

For decades vaccines have been proven invaluable in the fight against major infectious diseases. Despite their efficacy, limited information is available on the precise immunological mechanisms exploited by universally used vaccines (Querec et al., 2006). We investigated 15 commonly used prophylactic vaccines for their immune modulating capability and their subsequent downstream effects on pDCs (Kadowaki et al., 2001). In man pDCs have a restricted TLR expression profile (TLR7 and TLR9) and, because of their secretion of high levels of IFN-α, have the ability to link innate and adaptive immunity. Triggering of TLR9 by vaccines and CpG oligonucleotides could be assigned to the same signaling pathways (Fig. 4). The effect on pDC of the prophylactic vaccines for Rabies and Mumps/Measles/Rubella (MMR) corresponds with that of CpG-A, which binds to the early endosomes of TLR-9 and therefore induces IFN-α production by pDC and lacks the ability to induce pDC maturation. Recently, a fortified Rabies vaccine showed a better response due to the addition of CpG ODN (BW006), a CpG type B oligonucleotide responsible for pDC maturation (Wang et al., 2008). This indicates that the intrinsic CpG type A property of the vaccine is effectively upgraded by adding a synthetic CpG-B oligonucleotide. Recently, a huge list of vaccines was described that induced TLR activation, in all those cases this is due to addition of synthetic TLR ligands to the vaccine and not based on TLR activation by the vaccine itself (Kanzler et al., 2007). Completely in line is the finding that after hepatitis B prophylactic vaccination the antigen specific immunity is enhanced in the presence of CpG compared to the vaccine alone (Angel et al., 2008). Noteworthy is that the here tested hepatitis vaccine alone was not able to trigger a CpG-like response (Fig. 1).

Prophylactic vaccines for meningitis and tuberculosis, Act-Hib and BCG, mimic CpG-B and traffic to late endosomes resulting in sole maturation of pDC. BCG-primed mice demonstrated a better protection against a challenge with *Mycobacterium tuberculosis* when a booster of BCG proteins supplemented with CpG-A was given (da Fonseca et al., 2009). Again this shows that a combination of CpG, one attributed to the vaccine and one synthetic, has a synergistic effect. CpG-C is localized in both early- and late-endosomes and this location in specific organelles in pDCs resulting in both maturation and IFN-α production. Based on the same type of reactivity as CpG-C we speculate that components of the FSME vaccine also reach both types of endosomes and this may play an important role in determining the nature of the immune response. Activation of pDCs and subsequent secretion of IFN-α has been demonstrated to activate natural killer cell lysis of

tumor cells in vivo. Released tumor antigens will be phagocytosed by IFN-α-matured macrophages. Furthermore, IFN-α secreted by pDCs is known to promote cross-presentation of tumor antigens by myeloid DC and induce T and B cell activation and survival (Pulendran et al., 2008). Therefore, components such as FSME, with its dual effect on the immune response are extremely interesting for cancer vaccine development (Krieg, 2007). Moreover, newly developed prophylactic vaccines can now be tested and classified into CpG subclasses and based on this their reactivity can be predicted or be optimized by the addition of synthetic CpG components.

An obvious way to test the involvement of TLRs in the activation as seen with the vaccines is to use murine dendritic cells obtained from TLR knockout mice. However, murine DC show a complete other DC maturation profile when induced by the different vaccines (data not shown). This fits with the recent finding that in man nickel allergy is mediated via TLR4 while in mouse it is not (Schmidt et al., 2010). The currently valid paradigm is that TLR9 exclusively responds to unmethylated CpG DNA motifs found in certain bacteria and viruses (Krieg, 2002a). However recent findings indicated that the Yellow fever virus (YF-17D), a single-stranded RNA virus, induced DC activation was reduced in TLR9$^{-/-}$ DCs (Querec et al., 2006). This raised the question whether contaminating DNA was present leading to DC activation. To exclude this possibility Querec et al. (2006) showed that DNase treatment of the YF-17D virus had no effect on the ability to induce IFNα secretion by DCs. These data in combination with the finding by Kato et al. (2005) that the Newcastle Disease virus, another single-stranded RNA virus, also activated DCs through TLR7 and TLR9, support the notion that TLR9 may not only recognize unmethylated, CpG-rich DNA, but also other microbial or viral derived components. Support for non-classical triggering of TLRs comes from recent evidendence that Ni^{2+} ions bind to TLR4 at a different location than its natural ligand LPS but display the same effects (Schmidt et al., 2010). Differences in CpG motifs in RNA due to different propagation can be excluded because CpG RNA does not induce IFN-α (Sugiyama et al., 2005). Apart from this concept, it is also important to bear in mind that commonly used vaccines not only contain viral or bacterial derived products, but also cellular contaminants derived from the cell lines in which the vaccines were generated (Janeway, 1989; Matzinger, 1994; Querec et al., 2006). These co-passengers in vaccine preparations can potentially induce immune activation, at first glance ascribed to the viral or bacterial components. This could in part explain the observation that in our hands the vaccine Stamaril does not induce human pDC activation (Fig. 1A, 1B), whereas Yellow fever propagated in the SW480 colon carcinoma cell line and purified by sucrose gradient centrifugation appeared to evoke an abundant IFN response when incubated with PDCs (Pulendran, 2009; Querec et al., 2006). Those differences in propagation methods (chicken eggs versus a cell line) apparently can induce different TLR triggering. Since Yellow fever binds to heparin sulfate glycoaminoglycans (Germi et al., 2002), the different glycosaminoglycan make-up of the human colon cancer cell line versus the chicken cells could cause this difference.

To the best of our knowledge, we show for the first time that vaccines, apart from their antigen content, have a very explicit action on the immune system and behave remarkably similar as synthetic CpG-ODN-subclasses. This knowledge and the fact that vaccine preparations are cheap, safe and readily available may revitalize and broaden interest in the use of commonly used vaccines as adjuvants in vaccination studies aimed at the treatment of cancer, allergy and autoimmunity (Krieg, 2007; Pulendran, 2007).

Conflict of interest

All authors declare no conflict of interest.

Author contributions

The experiments were designed by IJMdV, JT, RT, CGF and carried out by JT and DB-R. The manuscript was written by IJMdV, RT, JT and CGF. The principal investigator is IJMdV.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molimm.2010.12.022.

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