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Bacterial DNA and CpG-Containing Oligodeoxynucleotides Activate Cutaneous Dendritic Cells and Induce IL-12 Production: Implications for the Augmentation of Th1 Responses

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Key Words

Dendritic cells · DNA · Langerhans cells · IL-12 · Oligodeoxynucleotides

Abstract

Background: Unmethylated CpG sequences in bacterial DNA act as adjuvants selectively inducing Th1 predominant immune responses during genetic vaccination or when used in conjunction with protein Ag. The precise mechanism of this adjuvant effect is unknown. Because dendritic cells (DC) are thought to be crucially involved in T cell priming and Th1/Th2 education during vaccination via skin, we characterized the effects of bacterial DNA and CpG-containing oligodeoxynucleotides (CpG ODN) on cutaneous DC. ***Methods and Results:*** Stimulation with CpG ODN 1826 (6 μg/ml) induced activation of immature Langerhans cell (LC)-like DC as determined by an increased expression of MHC class II and costimulatory molecules, loss of E-cadherin-mediated adhesion and increased ability to stimulate allogeneic T cells. Composition-matched control ODN 1911 lacking CpG sequences at equal concentrations was without effect. In comparison to LPS and ODN 1911, CpG ODN 1826 selectively stimulated DC to release large amounts of IL-12 (p40) and little IL-6 or TNF-α within 18 h and detectable levels of IL-12 p70 within 72 h. Stimulation with *Escherichia coli* DNA, but not calf thymus DNA, similarly induced DC maturation and IL-12 p40 production. Injection of CpG ODN into murine dermis induced enhanced expression of MHC class II and CD86 by LC in the overlying epidermis and intracytoplasmic IL-12 p40 accumulation in a subpopulation of activated LC. ***Conclusion:*** Bacterial DNA and CpG ODN stimulate DC in vitro and in vivo and may preferentially elicit Th1-predominant immune responses because they can activate and mobilize DC, inducing them to produce IL-12.

Introduction

Bacterial DNA can act as Th1 promoting adjuvants during genetic vaccination or when used in combination with protein antigen [1–5]. The adjuvant properties of bacterial DNA are related to the high frequency of unmethylated CpG dinucleotides in bacterial as compared with eukaryot-

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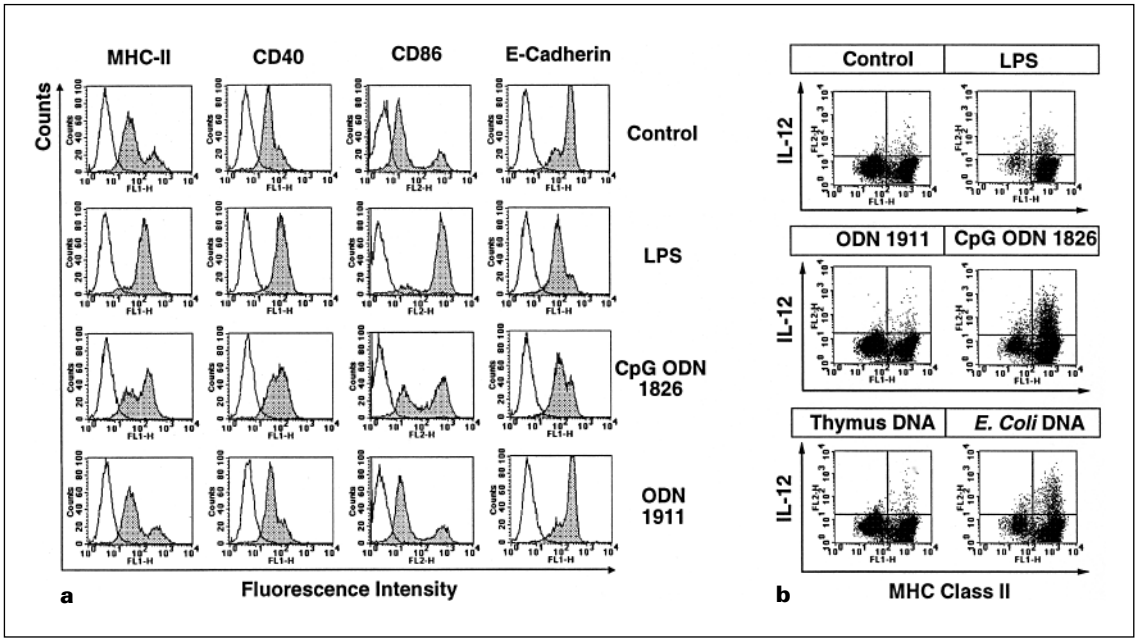

Fig. 1. Activation of LC-like FSDDC by CpG ODN 1826 and bacterial DNA. **a** Enhanced expression of MHC and costimulatory molecules and loss of E-cadherin expression by FSDDC treated with CpG ODN 1826. FSDDC were stimulated with LPS (100 ng/ml) or ODN (6 μg/ml) for 18 h and analyzed for expression of the indicated Ag using flow cytometry. Shaded areas represent antibody of interest and the solid line isotype control. **b** Induction of FSDDC IL-12 production by CpG-containing ODN and bacterial DNA. FSDDC were treated with LPS (100 ng/ml), ODN 1911 or CpG ODN 1826 (6 μg/ml), calf thymus DNA or *E. coli* DNA (30 μg/ml) and surface MHC class II Ag and intracellular IL-12 p40 were simultaneously quantitated using flow cytometry.

ic DNA [reviewed in 6] and can be mimicked by synthetic oligodeoxynucleotides (ODN) containing a consensus immunostimulatory motif (5′-purine-purine-CpG-pyrimidine-pyrimidine-3′; CpG ODN [7]. Because dendritic cells (DC) are thought to be essential for the initiation of primary immune responses in T cells in vivo [8], and DC-derived cytokines influence T helper cell differentiation [9], we hypothesized that bacterial DNA and CpG ODN might be potent activators of DC. We assessed the response to bacterial DNA and CpG ODN using an in vitro model of murine epidermal Langerhans cells (LC) [fetal skin-derived DC (FSDDC)] that has recently been developed in our laboratory [10]. FSDDC were particularly suitable for these studies, because they display a relatively stable immature phenotype and can be induced to aquire the phenotype and function of mature interdigitating DC by stimulation with known agonists of DC maturation [11]. We also assessed ODN for LC-activating activity in vivo.

Materials and Methods

Murine FSDDC were propagated from day 16 C57BL/6 fetal skin and isolated as aggregates as recently described [10]. FSDDC aggregates were subcultured at 5×10^5 cells/ml in the presence or absence of DNA, ODN and LPS as indicated. CpG containing ODN (CpG ODN 1826 5′-TCCATGACGTTCCTGACGTT-3′) and composition-matched control ODN (ODN 1911 5′-TCCAGGACTTTCCTCAGGTT-3′) with a phophorothioate backbone were synthesized by Oligos Etc. (Wilsonville, Oreg.). *Escherichia coli* and calf thymus DNA were purchased from Sigma Chemical Co. (St. Louis, Mo.) and prepared for use as described [12]. Cells were stained for surface Ag expression [10] and intracellular IL-12p40 as previously described and analyzed using a FACScan flow cytometer (Becton Dickinson, Mountainview, Calif.) [12]. Cytokine release into 18 and 72 h FSDDC supernatants was measured as described using ELISAs specific for IL-1β, IL-12 p70 (Genzyme, Cambridge, Mass.), IFN-γ, TNF-α, IL-6 and IL-12 p40 [12].

To assess the activation of LC in vivo, dosal ear skin of anesthetized mice was injected with recombinant murine IL-1β (50 ng in 50 μl, Genzyme Diagnostics, Cambridge, Mass.) or ODN (50 mg in 50 μl PBS). After 12 h, epidermal cell suspensions were obtained [12] and the surface phenotype of LC was characterized using flow cytometry. Intracellular IL-12 p40 accumulation in LC was evaluated in epidermal cell suspensions obtained 12 h after injection and an additional 5-hour culture in complete media supplemented with brefeldin A (1 μg/ml).

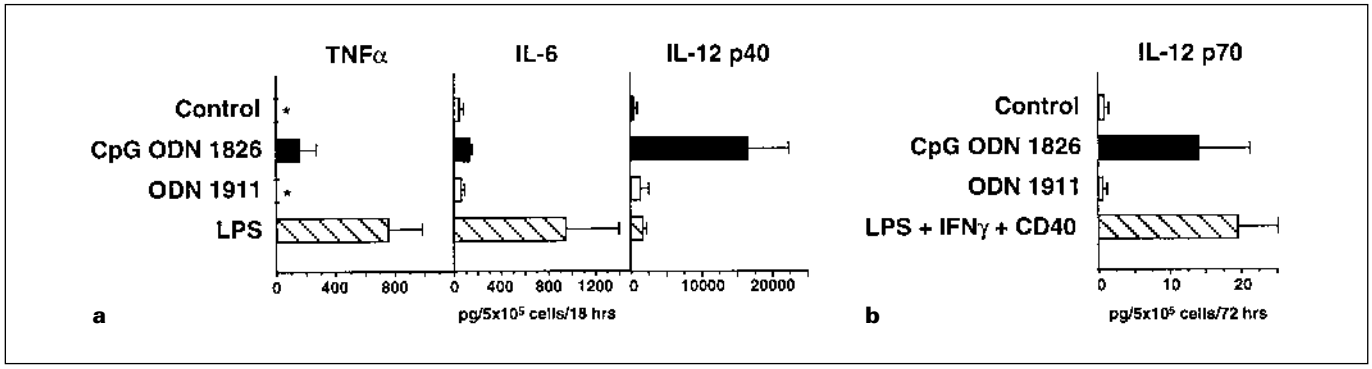

Fig. 2. Induction of FSDDC cytokine production by CpG ODN. **a** FSDDC were incubated for 18 h in presence or absence of LPS (100 ng/ml) or ODN (6 μg/ml) and supernatants were analyzed for cytokines. **b** IL-12 p70 production was determined after 72 h of stimulation with either ODN (6 μg/ml) or a combination of LPS (100 ng/ml), IFN-γ (1,000 U/ml) and anti-CD40 mAb (10 μg/ml).

Results

CpG ODN Induce DC Maturation and DC IL-12 Production

We previously demonstrated that immature DC expanded in primary cultures of murine fetal skin cells (FSDDC) and isolated as trypsin-resistant aggregates resemble epidermal LC [10]. Adhesion within aggregates is E-cadherin-mediated [10], and serves as an in vitro correlate of E-cadherin-mediated adhesion of LC and keratinocytes in epidermis [13]. Known activators of DC maturation (e.g. LPS, IL-1, TNF-α) induce loss of adhesion in FSDDC aggregates and initiate maturation which correlates with their ability to activate and mobilize LC in vivo [11]. Stimulation of FSDDC with CpG ODN 1826 (6 μg/ml) induced a similar maturation with loss of E-cadherin surface expression [accompanied by loss of cell adhesion, 12] and dramatically increased the expression of MHC class II Ag, CD40 and CD86 (see fig. 1a). Identical concentrations of control ODN 1911 did not result in significant changes in cell surface phenotype.

CpG ODN 1826 also induced FSDDC to produce within 18 h ~5-fold less TNF-α, ~7-fold less IL-6 and ~10 times more IL-12 as compared to LPS (fig. 2a). Staining for intracellular IL-12 p40 confirmed FSDDC IL-12 production at the single cell level and demonstrated that bacterial DNA and CpG ODN but not mammalian DNA or control ODN preferentially induced IL-12 p40 accumulation in FSDDC that displayed the most mature phenotype (fig. 1b). Consistent with previous reports [14], IL-12 p70 was detected only after 72 h of stimulation (fig. 2b). Note that IL-12 p70 release induced by CpG ODN 1826 was comparable to that obtained only after maximal stimulation with a combination of LPS, IFN-γ and CD40 ligation. CpG ODN-induced changes in cell surface phenotype and cytokine production were also accompanied by an increase in the ability of FSDDC to stimulate unprimed T cells, as determined in a mixed leukocyte reaction [12].

CpG ODN Activate LC in vivo

Introduction of CpG ODN 1826 (but not ODN 1911) into murine skin led to increased expression of MHC class II and CD86 Ag by significant subpopulations of LC obtained from skin overlying the injection site (see fig. 3a). Injection of the known agonist IL-1β served as positive and PBS as negative controls. Intracellular IL-12 accumulation was assessed in LC from BALB/c ears injected with LPS or CpG ODN 1826. A small subpopulation of LC activated by CpG ODN 1826 in vivo (4.3±1.6%, n = 3) contained detectable levels of intracellular IL-12 whereas LPS-activated LC did not (fig. 3b).

Discussion

The present study demonstrates that bacterial DNA and CpG ODN activate immature cutaneous DC in vitro and in vivo and induce DC IL-12 production. The effects of CpG ODN 1826 are dependent on the immunostimulatory motif, since the base composition-matched control ODN 1911 lacked activity when used at the same concentration (6 μg/ml). When studied in more detail [12] higher concentrations

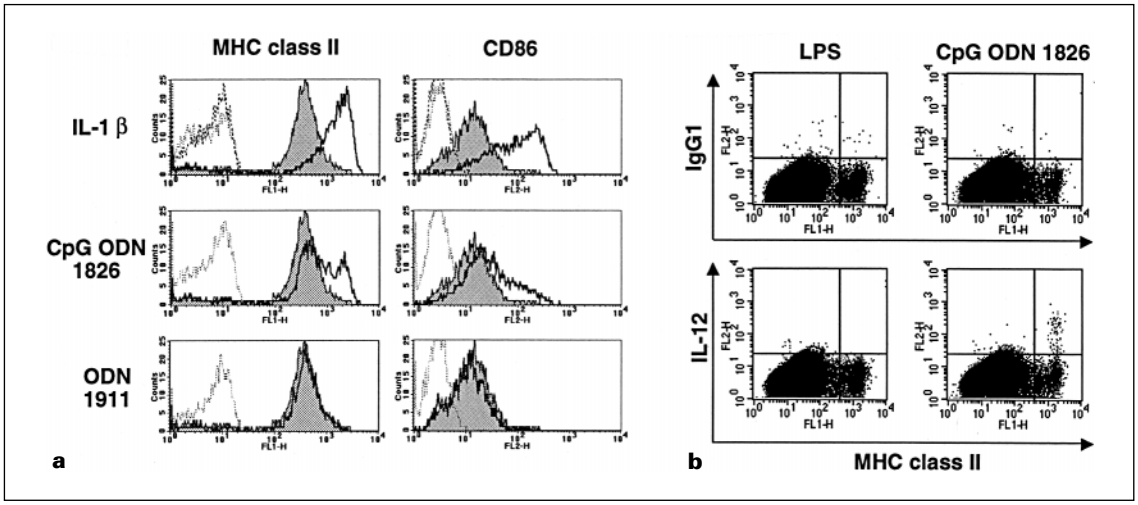

Fig. 3. In situ activation of LC by CpG ODN 1826. **a** Epidermal cell suspensions were prepared from BALB/c ear skin overlying sites injected 12 h earlier with IL-1β (50 ng), CpG ODN 1826 (50 μg) or PBS, stained for simultaneous expression of MHC class II Ag and CD86 and analyzed by flow cytometry. Dotted line represents isotype control, shaded areas LC from PBS-injected skin stained with the mAb of interest, and solid line LC from IL-1β-or ODN-injected skin stained with mAb of interest. **b** Selective induction of IL-12 biosynthesis in LC after intracutaneous administration of CpG ODN 1826 (50 μg) but not after injection of LPS (200 ng).

of ODN 1911 (60 μg/ml) also activated FSDDC. Non-sequence specific effects of ODN were not related to the backbone modifications since phosphodiester ODN and sequence-matched phosphorothiate ODN had a comparable DC-activating potential [12]. Additional physicochemical properties common to anionic polymers [15] may be relevant for the non-sequence-specific effects of ODN.

Previous studies demonstrated that bacterial DNA and CpG ODN can activate B cells [7], NK cells [16] and macrophages [17–19]. The propensity of bacterial DNA or CpG ODN to induce Th1-predominant immune responses has previously be attributed to a CpG-dependent IL-12 production by macrophages. In order for a primary Th1 response to develop, IL-12 needs to be present at the time of Ag presentation [9, 20]. Since DC, rather than macrophages, are primarily responsible for T cell priming [8], we suggest that CpG-dependent activation of DC and induction of DC IL-12 promote the initial phases of Th1 differentiation during genetic vaccination. Similar scenarios may occur in the setting of chronic infections that likely result in the release of immunostimulatory DNA into skin, or in instances where CpG-containing DNA is coadministered with protein Ag [2, 3, 5]. The propensity of bacterial DNA and CpG ODN to promote the development of Th1 responses may have a potential application in various clinical settings. Recent studies suggest that CpG ODN-dependent induction of Th1 immunity may be beneficial in the treatment of cancer [4] or the prevention of infectious diseases [5]. Finally, CpG ODN may also be useful in the downregulation of an ongoing Th2 response, as recently reported in a murine model of allergic asthma [21].

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