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Short communication

Intranasal delivery of influenza antigen by nanoparticles, but not NKT-cell adjuvant differentially induces the expression of B-cell activation factors in mice and swine

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ABSTRACT

Intranasal vaccination of pigs with poly lactic-co-glycolic acid and polyanhydride nanoparticles delivered inactivated influenza virus provides cross-reactive T-cell response, but not antibody response, resulting in incomplete protection and no reduction in nasal virus shedding. Expression of BAFF and Th2 transcription factor GATA-3 were downregulated in lungs of pigs vaccinated with influenza nanovaccine, but in mice it upregulated the expression of BAFF and cytokine TGFβ in cervical lymph nodes. However, the intranasal iNKT cell adjuvant, α-Galctosylceramide upregulates the expression of BAFF in pig lungs. In conclusion, expression of BAFF is differentially regulated by intranasal nanovaccine and α-Galctosylceramide in pig respiratory tract.

1. Introduction

Soluble antigens are poorly immunogenic but when entrapped in polymer-based nanoparticles (NPs) become highly immunogenic [1]. The NPs (< 500 nm) entrapped soluble antigens are efficiently internalized, processed and presented by antigen presenting cells (APCs) [2]. One of the widely used FDA approved, safe, nontoxic, synthetic biodegradable polymer in drug delivery is poly lactic-co-glycolic acid (PLGA) [3]. In mice PLGA NPs-entrapped influenza A virus (IAV) hemagglutinin (HA) [4] induces higher antibody response than did soluble antigen. Another synthetic polymer, polyanhydride NPs delivered viral nanovaccine in mice induces germinal center B-cell formation and serum antibody response [5]. Intranasally-delivered polyanhydride-based IAV HA vaccine induces virus neutralizing antibody and cell-mediated immune responses and protects mice against H5N1 challenge infection [6]. However, in pigs intranasal delivery of PLGA NPs or polyanhydride NPs based IAV vaccine upregulates T-cell response and but not mucosal IgA and serum IgG responses [7,8]. This suggests that intranasal PLGA and polyanhydride-based influenza nanovaccine in pigs and mice differentially activate B-cells.

The TNF superfamily molecules, B-cell activation factor (BAFF) and a proliferation-inducing ligand (APRIL) are critical for survival, maintenance, activation and maturation of B- cells [9]. APRIL plays a role in IgA response as evidenced by impaired IgA class switching in APRIL-deficient mice [10]. TGFβ1 acts as a B-cell isotype-specific switch factor for IgA production. Activation-induced cytidine deaminase (AID) is a member of the RNA-editing deaminase family in B-cells of germinal centers, and it is required for immunoglobulin somatic hypermutation and class switch recombination in T-cell dependent immune responses [11]. The B-cell activation associated factors are shown in a schematic diagram (Fig. 1). In pigs, BAFF and APRIL are known to facilitate B-cell function [12]. Pigs, like humans are natural hosts for influenza, and development of potent intranasal influenza vaccines in the pig model will have duel benefits for both humans and swine populations [13].

Invariant Natural killer T (iNKT) cell is a specialized T lymphocyte subset, having unique ability to modulate both innate and adaptive immune responses against pathogens through its ability to rapidly secrete copious amounts of Th1, Th2 and Th17 cytokines upon activation by unique glycolipid antigens such as α-Galctosylceramide (α-GalCer) [14]. This study was undertaken to elucidate the expression of factors

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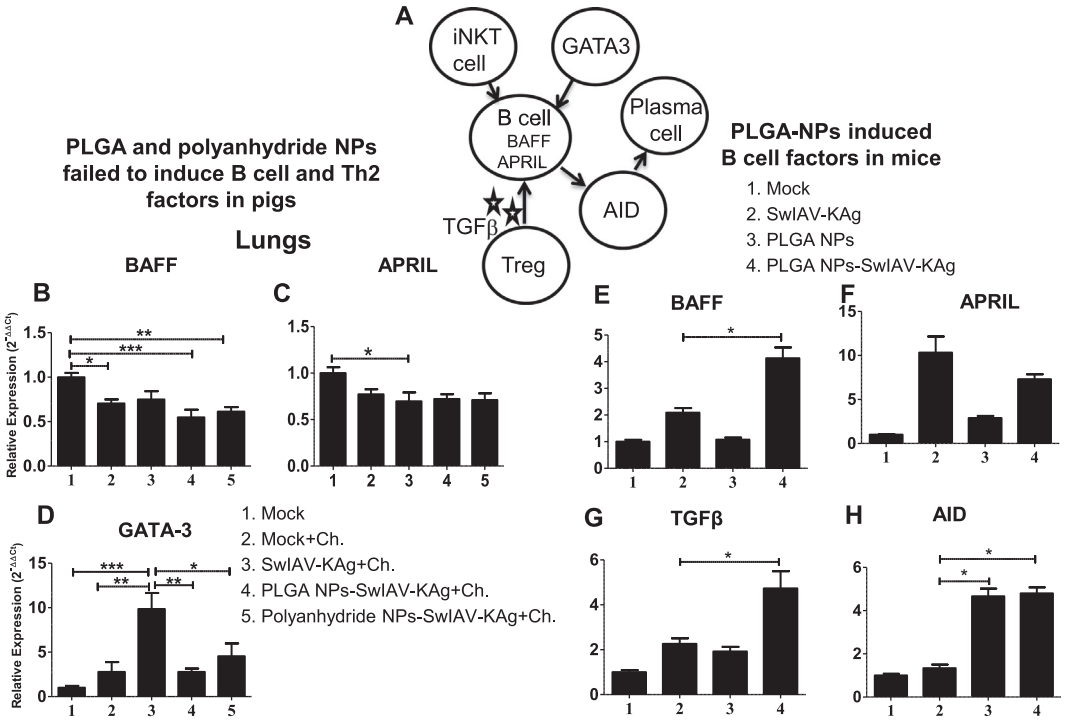

Fig. 1. Expression of B-cell activation factors in the lungs and lymph nodes of pigs and mice to IAV nanovaccine. Pigs were vaccinated twice IN with inactivated swine IAV either in soluble form or entrapped in PLGA NPs or in polyanhydride NPs and then challenged with a heterologous IAV two weeks after the booster. (A) Schematic of the B-cell activation associated cells and factors. Pigs were euthanized on day 6 post-challenge. BAL cells were analyzed for the expression of mRNA of (B) BAFF, (C) APRIL and (D) Th2 transcription factor GATA-3 by RT-qPCR. Mice were vaccinated once IN with inactivated swine IAV in soluble or in PLGA NPs and euthanized 24 h later. Cervical lymph nodes were analyzed for the expression of mRNA of (E) BAFF, (F) APRIL, (G) TGFβ and (H) AID by RT-qPCR. The relative expression of mRNA of indicated genes in BAL cells of pigs (n = 7 or 9) and cervical lymph nodes of mice (n = 3) were normalized with β-actin. Each bar is the mean ± SEM values in each animal group. Asterisk denotes statistically significant difference ($^{*}P < .05$, $^{**}P < .001$, $^{***}P < .0001$) between the indicated two groups. Ch: challenge; PLGA: poly lactic-co-glycolic acid; NPs: Nanoparticles; SwIAV: Swine influenza A virus; KAg: Killed antigen.

associated with B-cell responses to intranasal influenza nanovaccine in mice and pigs, and intranasal iNKT cell adjuvant α-GalCer in the respiratory tract of pigs.

2. Materials and methods

2.1. Pigs and mice

Frozen BAL cells and tonsils of the two previous studies conducted using PLGA and polyanhydride NPs delivered swine IAV antigens intranasally (IN) in pigs [7,8,15] were used in this study. C57BL/6 mice (The Jackson Labs, Bar Harbor, ME) were used at 9–12 weeks of age. Mock PBS, PLGA NPs or 20 μg of soluble swine IAV antigen unentrapped or entrapped in PLGA NPs were delivered IN, 20 μl per mouse once, euthanized 24 h later and cervical lymph nodes were used for gene expression analysis. To adjust the required amount and anatomic architecture of the upper respiratory tract of mice and pigs, the dose of NPs based swine IAV used in mouse was 0.081 times the pig and human dose as described previously [7,8,16]. All the animal studies were carried out in accordance with the approved protocol of the Institutional Animal Care and Use Committee of The Ohio State University.

2.2. Reverse transcriptase quantitative PCR (RT-qPCR) analysis

Total RNA was extracted from BAL cells and tonsils of pigs and cervical lymph nodes of mice using TRIzol reagent (Invitrogen). The expression of levels of mRNA of B-cell activation factors and cytokines was determined by RT-qPCR as described previously [17] and using the primers set listed in Table 1.

Table 1
Sequence of the primers used for RT-qPCR analyses of pig and mouse samples.

	Oligo name	Pig Sequences (5′→3′)
1	β-actin	CAGCCTCCTGAAACTGGAATAT (F) TCAGCAACAAGGTCTACAATCC (R)
2	BAFF	GAGAGCAGCTCCATTCAAAG (F)GCATGCCACTGTCTGCAATC (R)
3	APRIL	TGCTCACCCGTAAACAGAAG (F)TAAACTCCAGCATCCCAGAC (R)
4	GATA-3	CCCGTCCTACTACGGAAAC (F)GTGGTGGATGGACGTCTTG (R)
	Oligo name	Mouse Sequences (5′→3′)
1	β-actin	GCG CAA GTA CTC TGT GTG GA (F) GAA AGG GTG TAA AAC GCA GC (R)
2	BAFF	AGG CTG GAA GAA GGA GAT GAG (F) CAG AGA AGA CGA GGG AAG GG (R)
3	APRIL	GGG GAA GGA GTG TCA GAG TG (F) GCA GGG AGG GTG GGA ATA C (R)
4	TGFβ	CCC TAT ATT TGG AGC CTG GA (F) CTT GCG ACC CAC GTA GTA GA (R)
5	AID	CCA GAC TTT GGG TCG TGA AT (F) TGG CTT GTG ATT GCT CAG AC (R)

2.3. Statistical analyses

Data are represented as the mean ± standard error of mean (SEM) of 3, 7 or 9 animals in each group. Data were analyzed by one way ANOVA followed by Tukey's post-hoc comparison test using the Graphpad Prism 5 (Graphpad software, CA). A p value less than .05 was considered statistically significant.