

6-8) RSV, RSV, Th1, Th2, RSV, RNA, paramyxoviridae family, A B strain, protein Th1, IL-5, IL-13, G protein, formaline inactivated RSV, Th2, IL-4, IL-5, IL-13, RSV, (goblet cell hyperplasia), IFN- γ , 13-18, 1960, formaline inactivated RSV, RSV, 80%, 19, RSV, DNA, plasmid DNA, DNA, Cytosine-phosphate-Guanine Oligodeoxynucleotide (CpG ODN), RSV, CpG ODN, RSV, 1. RSV, 1, BALB/c, specific, pathogen free, RSV, human RSV A2, (catalogue no. VR 1302), RSV, HEp-2 cell, (Eagle's minimum essential medium (Gibco) supplemented with 2% fetal bovine serum), HEp-2 monolayer, RSV, inoculation, 80%

harvest, 18°C, 2000 g, pellet, 15, (10 mmol/L PBS containing 15% sucrose), -70°C, microplaque immunoperoxidase method, 18, 8, Sham, control ODN, CpG ODN, RSV, 1, (3-5) BALB/c, 10 μ L, RSV (1 \times 10⁶ pfu), RSV, 2, Control ODN, 5-TGACTGTGAAGGTTGGAGATGA-3 (GenoTech, Daejeon), CpG ODN, 5-TGACTGTGAACGTTCCGAGATGA-3 (GenoTech, Daejeon), 2 μ g, 5, (6), RSV, 4, (Fig. 1).¹⁸, 2., RSV, methacholine (Mch), 3.1 mg/mL, 50 mg/mL, nebulizer (Omron, Bannockburn, USA), forced oscillometer (Flexivent system; SCRIBQUE, Quebec, Canada), pento-barbital (100 mg/kg), 18 gauge ventilator, (tidal breathing - 25.0 mL/kg, - 120 /min), RSV, Mouse age: 1 wk, 6 wks, Protocol day: 0 2 d, 5 wks, 5 wks+4 d, ODN, Analysis, Groups: Sham, Control ODN, CpG ODN

Fig. 1. Schematic of study protocol and exposure groups. Neonatal mice (within 1 weeks of age) were infected with RSV (1 \times 10⁶ pfu) and re-infected 5 weeks after primary infection (6 weeks of age) and followed by assessment of airway function, airway inflammation, and lung histopathology 4 days after RSV reinfection. CpG ODN (1,826 s; 2 mcg/mouse) or control ODN were also administered intranasally 2 days after initial RSV infection.

3. (BAL fluid) Hank's Balanced Saline
 1 mL
 cytopsin (100 USA), Diff-Quick (IMEB Inc, CA, 300), 10% formaline, H&E PAS, paraffin block

4. RSV IgG2a
 BAL fluid IFN- γ , IL-13, IL-5, R&D systems (TECHNE Corporation, Minneapolis, USA)
 ELISA RSV IgG2a (RSV-IgG2a) RSV 2 μ L/mL, ELISA plate well 100 μ L 4 $^{\circ}$ C 24
 horseradish peroxidase (HRP) conjugated monoclonal rat anti-mouse IgG2a (SouthernBiotech, Birmingham, AL, USA) 100 μ L 1.5. Substrate (p-nitrophenylphosphate, 1 mg/mL in diethanolamine buffer, pH 9.8), microplate reader (Molecular Devices, CA, USA) 450 nm ELISA well

5. SPSS version 11.0 for Windows

95%
 1. 25 mg/mL Sham
 1.0425 \pm 0.1474 cmH₂O/mLsec, Control ODN 1.9100 \pm 0.1372 cmH₂O/mLsec, CpG ODN 1.2080 \pm 0.1337 cmH₂O/mLsec
 50 mg/mL Sham 1.1725 \pm 0.1089 cmH₂O/mLsec, Control ODN 3.6360 \pm 0.2475 cmH₂O/mLsec, CpG ODN 1.7940 \pm 0.2541 cmH₂O/mLsec
 Control ODN, CpG ODN
 Control ODN (P < 0.01, Fig 2A).
 (compliance) 25 mg/mL Sham 0.0332 \pm 0.0043 mL/cmH₂O, Control ODN 0.0166 \pm 0.0009 mL/cmH₂O, CpG ODN 0.02895 \pm 0.0025 mL/cmH₂O
 50 mg/mL Sham 0.0315 \pm 0.0025 mL/cmH₂O, Control ODN 0.0095 \pm 0.0008 mL/cmH₂O, CpG ODN 0.0199 \pm 0.0041 mL/cmH₂O
 Control ODN (P < 0.05, Fig 2B).

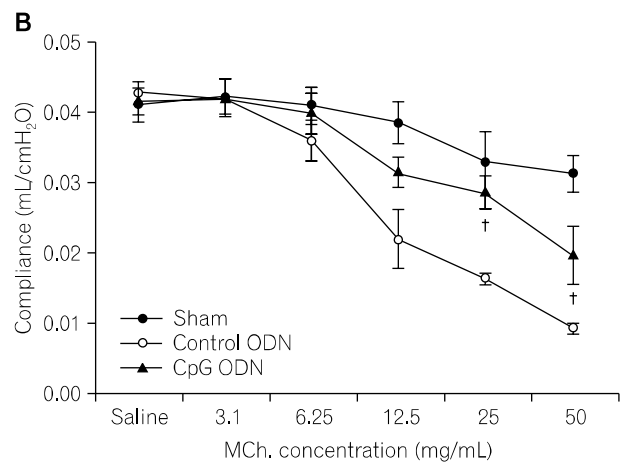
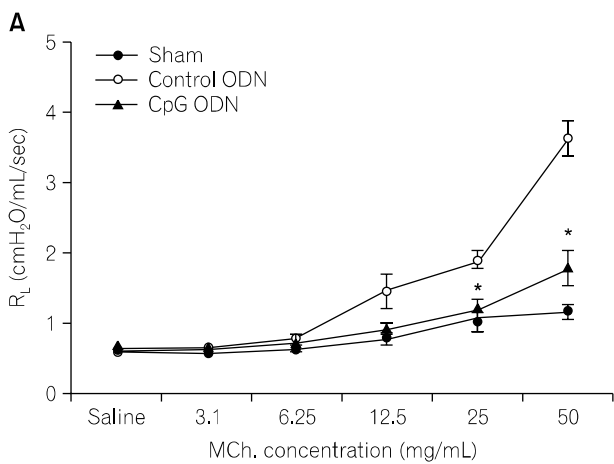


Fig. 2. Airway hyperresponsiveness develops after RSV re-infection and is not affected by administration of control ODN but affected by CpG ODN. There were differences between sham, control ODN, and CpG ODN group in airway resistance (A) and dynamic compliance (B) (*P < 0.01; † P < 0.05).

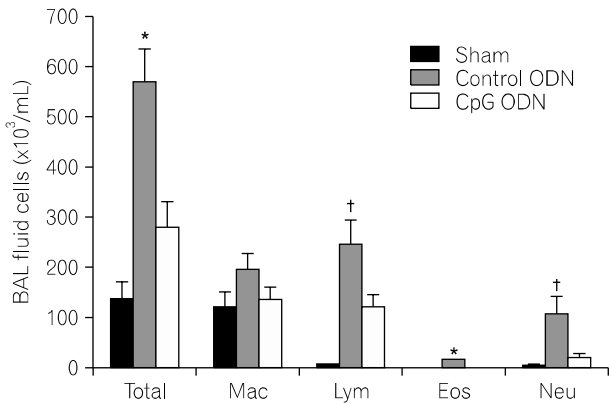


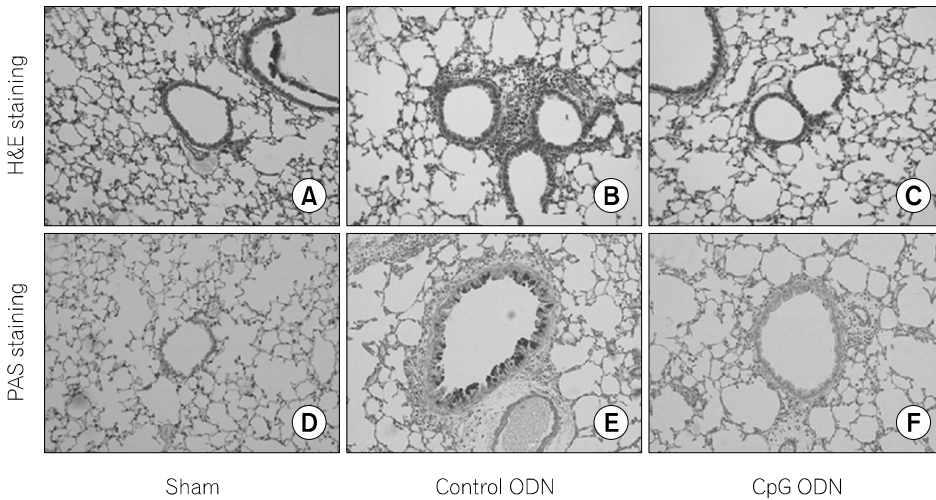
Fig. 3. Bronchoalveolar lavage (BAL) fluid cell counts. The results are from the same mice shown in Fig. 2. There were differences in BAL fluid lymphocyte (Lym), neutrophil (Neu) and eosinophil (Eos) counts between the control ODN and CpG ODN group (* $P < 0.01$; † $P < 0.05$).

2.

RSV

sham	140.0 ± 32.9 × 10 ³ cells/mL	CpG ODN	283.3 ± 50.6 × 10 ³ cells/mL
Control ODN	570.0 ± 66.4 × 10 ³ cells/mL	Control ODN	199.2 ± 28.4 × 10 ³ cells/mL
sham	123.0 ± 29.8 × 10 ³ cells/mL	CpG ODN	138.5 ± 27.1 × 10 ³ cells/mL
sham	7.5 ± 1.8 × 10 ³ cells/mL	Control ODN	123.7 ± 24.6 × 10 ³ cells/mL
CpG ODN	123.7 ± 24.6 × 10 ³ cells/mL	Control ODN	248.1 ± 46.4 × 10 ³ cells/mL
sham	0.2 ± 0.2 × 10 ³ cells/mL	CpG ODN	0.5 ± 0.3 × 10 ³ cells/mL
Control ODN	16.4 ± 3.7 × 10 ³ cells/mL	Control ODN	16.4 ± 3.7 × 10 ³ cells/mL
sham	6.8 ± 3.3 × 10 ³ cells/mL	CpG ODN	23.0 ± 7.5 × 10 ³ cells/mL
Control ODN	109.8 ±		

($P < 0.01$), ($P < 0.05$), ($P < 0.01$)



Sham Control ODN CpG ODN

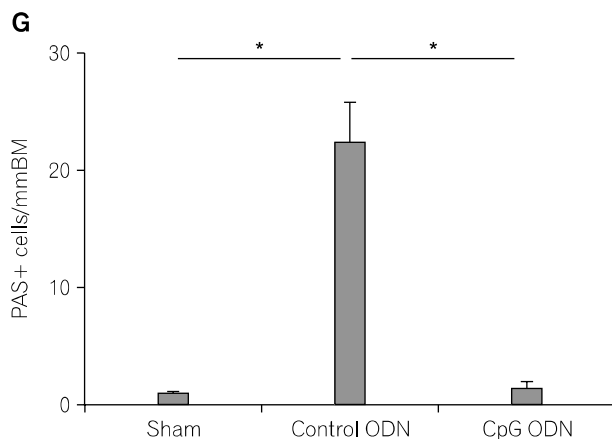


Fig. 4. Lung histologic features in respiratory syncytial virus (RSV)-reinfected mice (A–F) and quantitative analysis of periodic acid-Schiff (PAS)-positive cells in the central airways (G). Lung tissues were stained with H&E (A–C, ×400) and PAS (D–F, ×400). RSV reinfection causes peribronchial and perivascular inflammation and goblet cell hyperplasia in control ODN group (B, E, and G). In CpG ODN administration group, bronchial goblet cell hyperplasia was markedly decreased (C, F, and G). Results are expressed as the number of PAS-positive cells per millimeter of epithelial basement membrane (BM) (G) (* $P < 0.01$).

36.4 × 10³ cells/mL (P < 0.05, Fig 3).

3. PAS

RSV sham Control ODN
, CpG ODN
Control ODN (Fig 4).
PAS sham 0.48 ± 0.92
cells/mmBM CpG ODN 1.45 ± 1.52 cells/mmBM
Control ODN 21.75 ± 8.27 cells/mmBM
(P < 0.01, Fig 4G).

4.

IL-13

IL-13 Control ODN 8.2 ± 2.4
pg/mL sham 0.0 ± 0.0 pg/mL
(P < 0.01), CpG ODN 1.8 ± 0.8 pg/mL
(P < 0.05, Fig 5A).

5.

IL-5

IL-5 Control ODN 3,251.0 ±
610.7 pg/mL sham 2,344.0 ± 652.7 pg/mL CpG ODN
2,432.0 ± 326.4 pg/mL
(P < 0.05, Fig 5B).

6.

IFN-

IFN- Control ODN

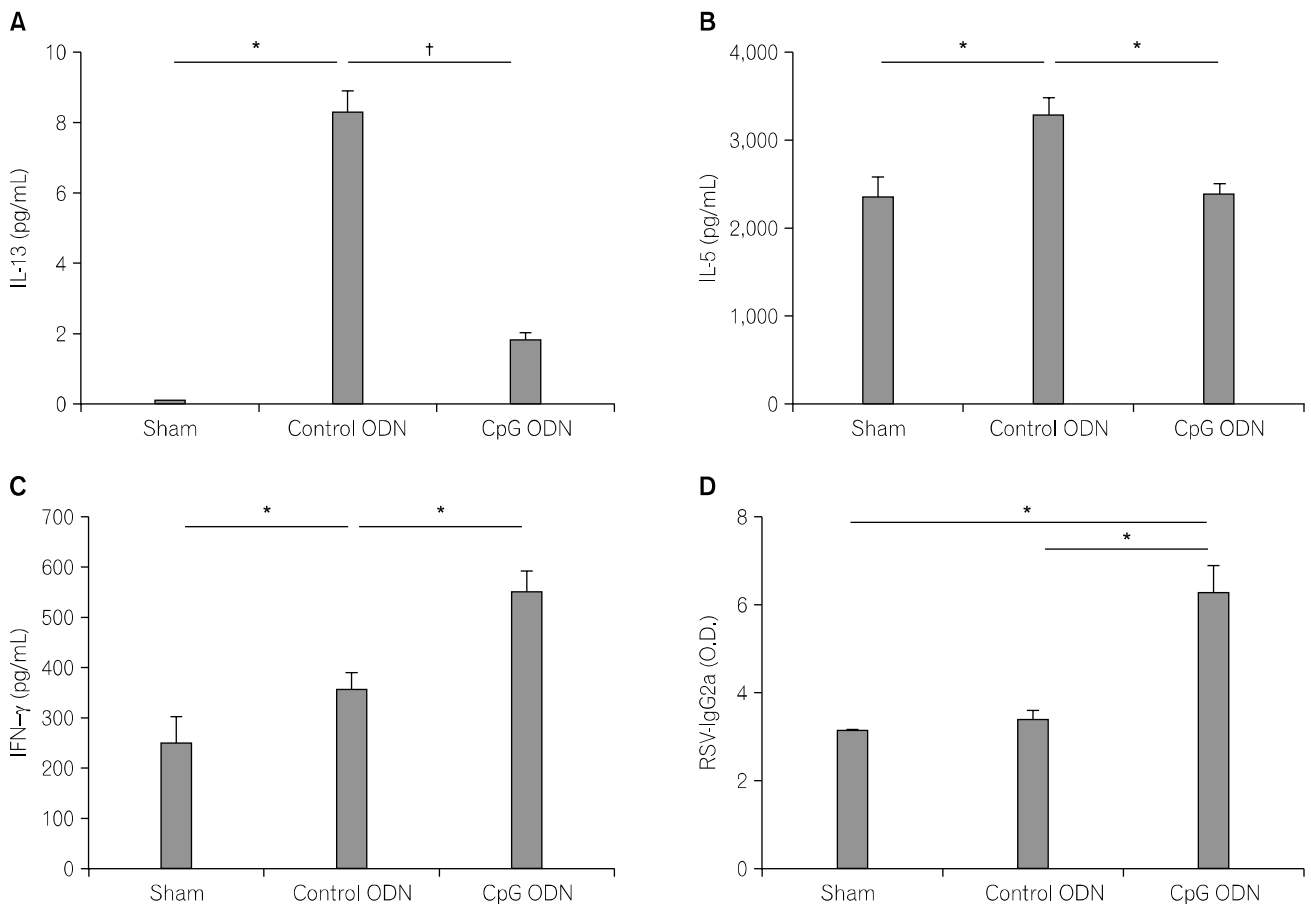


Fig. 5. Levels of cytokines in the bronchoalveolar lavage fluid (BAL) of RSV re-infected mice (A–C) and RSV-IgG2a in the sera of RSV re-infected mice (D). Level of IL-13 was lower in CpG ODN group than control ODN group (A). Level of IL-5 was increased in control ODN group, but the level of CpG ODN group was lower than control ODN group (B). Level of IFN- γ was more increased in CpG ODN group than in control ODN group (C). RSV-IgG2a of the sera was more increased in CpG ODN group than in other groups (D) (*P < 0.01; † P < 0.05).

355.0±149.4 pg/mL sham 225.0±162.7 pg/mL
 , CpG ODN 550.0±168.8 pg/
 mL (P<
 0.05, Fig 5C).

7. RSV IgG2a

RSV IgG2a (RSV-IgG2a) CpG ODN
 6.24±1.88 O.D. sham 3.09±0.36 O.D. Control ODN
 3.44±0.46 O.D. (P<
 0.05, Fig 5D).



RSV
 IL-4 IL-5 IL-13
 . RSV

1 2 strain RSV

.²⁰ RSV

RSV CD8+
 CD8+

IFN-

RSV

²²⁻²⁵⁾

RSV CD8+ T Th1 IFN-
 .²⁶⁾

RSV RSV
 . 3 RSV

RSV

, 1 RSV 6
 Th2

1 Th1 Th2 .¹⁸⁾ RSV
 RSV 1

B Th1 Th2 IgA IgG 1
 , RSV

RSV Th2 Th1 IFN- knock out mouse
 IL-4, IL-5, IL-13 histamine, prostaglandin

.²⁷⁾ Control ODN 1
 RSV 5 (6)
 Th2

DNA adjuvant 1937 Freund
 ,²⁸⁾ DNA

.¹⁹⁾ DNA CpG motif
 DNA CpG motif
 (5'-purine-purine-CG-pyrimidine-pyrimidine-3')

MHC
 class II CD80 CD86 costimulatory molecule
 , 4 IL-6 IL-12 Th2
 IL-4 IL-5 IL-13
 NK T Th1
 cytotoxic T Th1
 IFN- B IgE

IgM IgG2a

.²⁹⁾ CpG ODN IL-13 IL-5 Control ODN
 , Th1 IFN- CpG
 ODN Control ODN CpG
 ODN RSV-IgG2a Control ODN

. Sham IL-13

CpG ODN

Control ODN CpG ODN
 Th2 Th1

CpG ODN IFN- IL-12

cytokine CpG ODN

.³⁰⁾ CpG ODN 0.5µ g 1µ g 2µ g 4µ g 10
 µ g

CpG ODN

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