Vero/BC-F cells to cultivate for an additional 5 days. EGFP expression in the cells was analyzed by fluorescence microscopy. A hemagglutination (HA) assay was performed as previously described (Tsurudome *et al.*, 2008). Briefly, each 100 μl of hPIV2ΔF/EGFP and BPL-hPIV2ΔF/EGFP stock was serially 2-fold diluted in PBS and added into a U-type 96-well plate. After 100 μl of 1% guinea pig erythrocytes was added into each well, the plate was incubated for 2 hr at 4°C.

For hPIV2ΔF/EGFP titration, hPIV2ΔF/EGFP genomic RNA was isolated from hPIV2ΔF/EGFP viral stock, using a High Pure viral RNA kit (Roche Applied Science, Indianapolis, IN), according to the manufacturer's instructions. One microgram of the isolated viral RNA was reverse transcribed, using an hPIV2 *NP* gene-specific primer (5′-CAA CATTCAATGAATCAGT-3′) and SuperScript II reverse transcriptase (Invitrogen). Subsequently, quantitative real-time PCR was performed as described later.

A replication-incompetent vesicular stomatitis virus G glycoprotein (VSV-G)-pseudotyped retrovirus (ReV) was generated as described previously (Suzuki *et al.*, 2012) with minor modifications. Briefly, PLAT-gp cells (Kitamura *et al.*, 2003), which stably express *gag-pol* of Moloney murine leukemia virus, were grown to about 50% confluency and the medium was replaced with DMEM containing 10% heat-inactivated FBS and no blasticidin. PLAT-gp cells were transfected with plasmid encoding the *VSV-G* envelope protein driven by the cytomegalovirus (CMV) promoter and a retroviral vector pMX-EGFP, which lacks *gag-pol* and *env* (Onishi *et al.*, 1996), using FuGENE 6 (Roche Applied Science) according to the manufacturer's instructions. The medium was replaced every 24 hr and the supernatant was harvested 48, 72, 96, 120, and 144 hr after transfection. The supernatant harvested at each time point was filtered and concentrated by ultracentrifugation at 16,500 rpm for 90 min at 4°C. The virus was then resuspended in PBS and stored at 4°C. Viral titer was determined by flow cytometry as described below. NIH3T3 cells were plated in a 12-well plate in 2 ml of DMEM with 10% heat-inactivated FBS. After the viral stock was serially 10-fold diluted, NIH3T3 cells were infected with the virus in the presence of Polybrene (8 μg/ml; Sigma-Aldrich) for 6 hr at 37°C, followed by replacement with fresh medium. At 48 hr postinfection, the viral titer was calculated on the basis of the number of EGFP-positive cells as determined by flow cytometry.

Viral transduction into DCs

MoDCs and BMDCs (2×10^5 cells per well) were plated in a 24-well plate and infected either with hPIV2ΔF/EGFP or ReV/EGFP at various multiplicities of infection (MOIs) for 48 hr. DCs were harvested and washed with FACS (fluorescence-activated cell-sorting) buffer (PBS containing 2% FBS). EGFP expression in DCs was analyzed by flow cytometry.

Flow cytometry

MoDCs and BMDCs unstimulated or stimulated either with lipopolysaccharide (LPS; Sigma-Aldrich), hPIV2ΔF/EGFP, or BPL-hPIV2ΔF/EGFP for 48 hr were harvested, and Fc receptors were blocked with TruStain FcX (BioLegend, San Diego, CA) in FACS buffer for 5 min. DCs were then stained with respective antibodies and washed with FACS buffer. The antibodies used were as follows: phycoerythrin (PE)-conjugated anti-CD40, CD80, CD86, HLA-A, and H-2K^b antibodies, peridinin chlorophyll protein (PerCP)/Cy5.5-conjugated anti-HLA-DR and I-A/I-E antibodies, and allophycocyanin (APC)-conjugated anti-CD11c antibody. PE-, PerCP/Cy5.5-, and APC-conjugated subclass-matched antibodies were used as the isotype controls, respectively. All antibodies used for flow cytometry were purchased from BioLegend. Data were acquired on a FACSCalibur (BD Biosciences, San Jose, CA) and analyzed with CellQuest software (BD Biosciences).

ELISA

Culture supernatant was harvested from MoDCs and BMDCs unstimulated or stimulated either with LPS, hPIV2ΔF/EGFP, or BPL-hPIV2ΔF/EGFP for 48 hr and stored at −80°C until use. Human and mouse IL-6 and IL-12p40 were measured according to the manufacturer's instructions (eBioscience, San Diego, CA).

Quantitative real-time PCR

To determine hPIV2ΔF/EGFP titer and viral genomic RNA copy numbers, real-time PCR was performed to amplify hPIV2 *NP*-intergenic-*P* sequences. Briefly, one-tenth of the reaction solution containing the viral cDNA product was added to TaqMan gene expression master mix (Applied Biosystems/Invitrogen) containing the probe and primers, giving a final reaction volume of 20 μl. PCR assay was performed in triplicate on a StepOnePlus real-time PCR system (Applied Biosystems/Invitrogen), according to the manufacturer's instructions. StepOne software v2.1 (Applied Biosystems/Invitrogen) was used to analyze the real-time PCR data. Cycle conditions were set as follows: initial template denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 sec, and annealing/extension at 60°C for 1 min. Data were expressed as the numbers of hPIV2ΔF/EGFP *NP* gene copies. Primers used to measure hPIV2 genomic copy numbers were 5′-ACACACTCA TCCAGACAAATCAAAC-3′ and 5′-TGTGGAGGTTATCTG ATCACGAA-3′. The probe used was 5′-AAGCACCGG ATTTCTAACCCGTCCG-3′.

To detect viral transcripts, total RNA was extracted from MoDCs or BMDCs infected with hPIV2ΔF/EGFP for 48 hr, using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. cDNAs were synthesized from the same amounts of total RNA, using oligo(dT)$_{20}$ primer and SuperScript II reverse transcriptase. Subsequently, real-time PCR was performed with specific primers for the hPIV2 *NP* gene and for the human or murine *GAPDH* gene. Each reaction contained 10 μl of 2× Power SYBR green master mix (Applied Biosystems/Invitrogen), forward and reverse primers, and 2 μl of the cDNA product. Real-time PCR was performed in triplicate as described previously. Primers used to measure the transcripts of hPIV2 *NP*, human *GAPDH*, and murine *GAPDH* were 5′-ACCAGTATCAGTAGCAAAGC-3′ and 5′-TAGCGGTTTGCTAGCAAAGATC-3′, 5′-GTGAAGG TCGGAGTCAACGGA-3′ and 5′-GGTGAAGACGCCAGT GGACTC-3′, and 5′-CCCTTATTGACCTCAACTACATGGT-3′ and 5′-GAGGGGCCATCCACAGTCTTCTG-3′, respectively. Cycle conditions were described previously. The levels of hPIV2ΔF/EGFP *NP* transcripts in each species with

increased MOIs were measured by the $\Delta\Delta C_T$ method and normalized by the expression of each *GAPDH* reference gene. Data are shown as the relative levels of *NP* gene transcripts of hPIV2ΔF/EGFP.

Statistical analyses

All data are shown as means±SD. Significant differences among groups were evaluated by one-way analysis of variance (ANOVA), followed by the Bonferroni test with R software for Windows. $p<0.05$ was considered statistically significant.

Results

Transduction of hPIV2ΔF and ReV into MoDCs

To assess the transduction efficiency of hPIV2ΔF/EGFP, we infected MoDCs with hPIV2ΔF/EGFP or ReV/EGFP at various MOIs for 48 hr. Much stronger EGFP expression in hPIV2ΔF/EGFP-infected MoDCs was observed by fluorescence microscopy, compared with that in ReV/EGFP-infected MoDCs in similar replication-incompetent single-round infectious systems (Fig. 1A). The percentages of EGFP-positive MoDCs infected with hPIV2ΔF/EGFP were 59.9, 73.1, and 77.8% at MOIs of 25, 50, and 100, respectively, whereas those infected with ReV/EGFP were 4.4, 11.2, and 25.2% at MOIs of 25, 50, and 100, respectively (Fig. 1B). Moreover, the mean fluorescence intensity (MFI) of EGFP, reflecting the amount of EGFP protein, in MoDCs infected with hPIV2ΔF/EGFP was much higher than that with ReV/EGFP (Fig. 1C). These data indicate that hPIV2ΔF transduced an exogenous gene into MoDCs more efficiently than ReV at 48 hr after infection, unveiling the great potential of hPIV2ΔF for transient gene delivery.

Comparison of replication efficiency of hPIV2ΔF in MoDCs and BMDCs

Because previous studies demonstrated that replication/transcription of some hPIVs was suppressed in L929 murine fibrosarcoma cells (Ito *et al.*, 1989; Komada *et al.*, 2000), we used primary murine BMDCs to investigate whether the replication efficiency of hPIV2ΔF/EGFP and the expression level of EGFP were reduced. As shown in Fig. 2A, hPIV2ΔF/EGFP genomic RNA copy numbers were 3.5-, 5.9-, and 4.7-fold lower in murine BMDCs (C57BL/6) than in human MoDCs at MOIs of 25, 50, and 100, respectively, and no significant difference was observed between the mouse strains (C57BL/6 and BALB/c). This finding suggests that viral uptake and/or genomic RNA replication of hPIV2 in murine cells is less efficient than in human cells. We next investigated *NP* transcripts in BMDCs and MoDCs at each MOI, using the $\Delta\Delta C_T$ method of real-time PCR and using each species of *GAPDH* as a reference gene (Fig. 2B). The *NP* transcripts increased MOI dependently in both species of cells. Although we could not directly compare the quantity of *NP* transcripts between murine and human cells, the real-time PCR analysis using the same amount of the cDNA without reference genes showed similar reduction in *NP* transcripts in murine cells as in *NP* genomic copy numbers (data not shown). Next, we investigated the transduction efficiencies of hPIV2ΔF/EGFP into BMDCs (C57BL/6). The

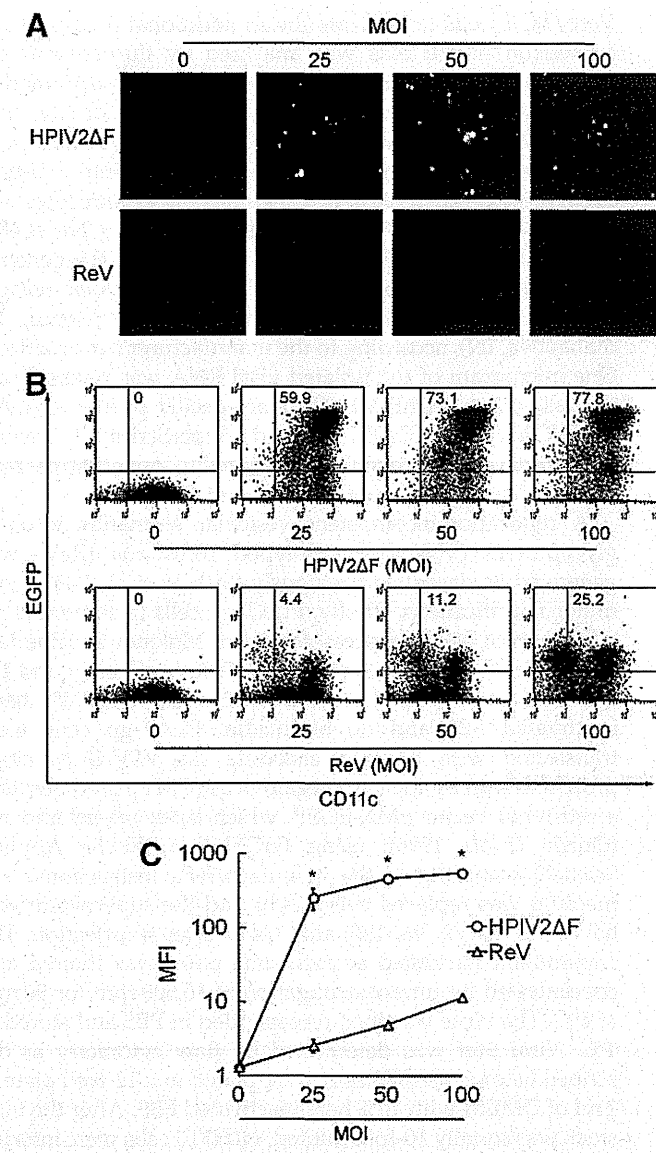

FIG. 1. Comparison of hPIV2ΔF and a retroviral (ReV) vector in terms of EGFP transduction efficiency in MoDCs at various MOIs 48 hr after infection. **(A)** EGFP expression in MoDCs was visualized by fluorescence microscopy. **(B)** The frequency of EGFP-positive MoDCs at various MOIs was determined by flow cytometry. **(C)** Mean fluorescence intensity (MFI) for EGFP expression in MoDCs at various MOIs. Data are shown as means±SD, and the statistical significance (*$p<0.05$) was determined by Bonferroni test.

percentages of EGFP-positive BMDCs were increased in an MOI-dependent manner and about 70% of BMDCs expressed EGFP at an MOI of 100 (Fig. 2C), but a significantly lower amount of EGFP was detected in hPIV2ΔF/EGFP-infected BMDCs by fluorescence microscopy (Fig. 2D) and by flow cytometry (data not shown) compared with those in human MoDCs. Consistent with the previously reported findings (Ito *et al.*, 1989; Komada *et al.*, 2000), the high MOI of hPIV2ΔF/EGFP was required to obtain appropriate transduction efficiency in the study using murine BMDCs to overcome the markedly reduced replication/transcription of hPIV2.

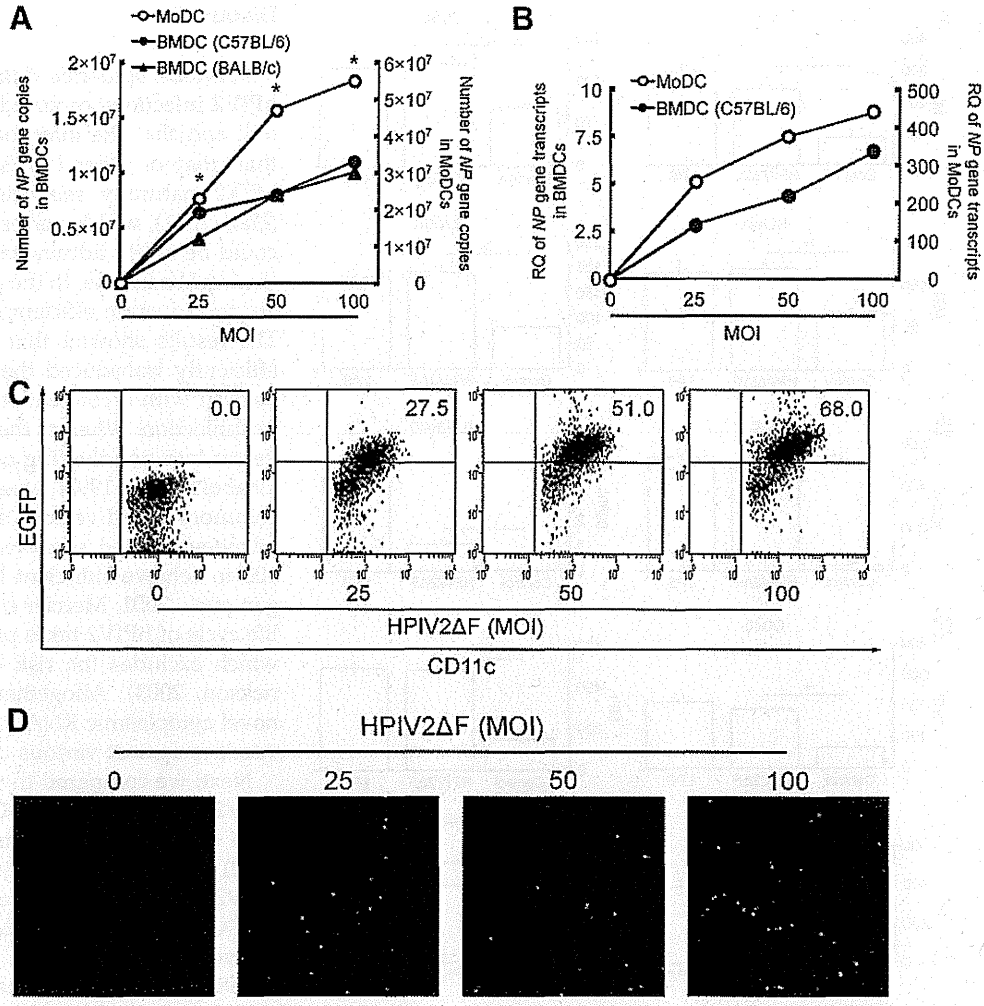

FIG. 2. HPIV2ΔF/EGFP shows low replication/transcription capacity in BMDCs. **(A)** BMDCs and MoDCs were infected with hPIV2ΔF/EGFP at various MOIs for 48 hr. The same amount of total RNA was reverse transcribed with the hPIV2 *NP* gene-specific primer, followed by real-time PCR. Shown are copy numbers of the hPIV2 genome evaluated by RT-PCR amplification of the *NP-P* region containing intergenic sequence. **(B)** Relative quantity (RQ) of hPIV2 *NP* transcripts in MoDCs and BMDCs. Shown are RQs of *NP* transcripts in MoDCs, relative to human *GAPDH* at each MOI, and that in BMDCs, relative to murine *GAPDH* at each MOI. **(C)** The percentages of EGFP-positive BMDCs infected with hPIV2ΔF/EGFP at various MOIs are shown in the upper right quadrant. **(D)** EGFP expression in BMDCs infected with hPIV2ΔF/EGFP at various MOIs was visualized by fluorescence microscopy.

Maturation states of MoDCs and BMDCs infected with hPIV2ΔF/EGFP

In *in vivo* DC-targeted vaccines and *ex vivo* DC therapies, not only transduction of vaccine antigens into DCs, but also DC maturation is pivotal to prime T cells. Thus, we investigated the effects of hPIV2ΔF/EGFP on DC maturation. Figure 3A shows surface expression of CD40, CD86, HLA-A, and HLA-DR on MoDCs stimulated with either hPIV2ΔF/EGFP or LPS, a well-known DC stimulator. In MoDCs, hPIV2ΔF/EGFP stimulation significantly increased the expression levels of their maturation markers, which were nearly comparable to those induced by LPS, compared with unstimulated MoDCs. High amounts of IL-6 and IL-12p40 were secreted from hPIV2ΔF/EGFP-stimulated MoDCs (Fig. 3B). These experiments were also performed in BMDCs (hereafter only C57BL/6 mice were used). Similar to MoDCs, hPIV2ΔF/EGFP-stimulated BMDCs significantly increased the expression of maturation markers and the amounts of cytokines (Fig. 3C and D). These results indicate that hPIV2ΔF/EGFP has strong stimulatory activity for both types of DCs. Interestingly, although the genomic copy numbers of hPIV2ΔF/EGFP in BMDCs were about 3.5 times lower than in MoDCs at an MOI of 25 (Fig. 2A), the expression levels of maturation markers on BMDCs and MoDCs after viral infection, relative to those after LPS stimulation, were nearly equal. These results suggest that DC maturation induced by hPIV2ΔF/EGFP depended not only on replication of the viral genome or *de novo* synthesis of viral proteins but also on preexisting hPIV2ΔF components (e.g., by binding of the envelope proteins to host receptors or by recognizing the original viral genome).

Effects of replication-defective hPIV2ΔF/EGFP on BMDC maturation

On the basis of the data described previously, we next investigated whether DC maturation was induced by preexisting viral components without replication/transcription. First, to generate replication-defective hPIV2ΔF/EGFP, we chemically inactivated hPIV2ΔF/EGFP. Because β-propiolactone (BPL) is a chemical reagent that has alkylating activity against adenosine and guanosine of viral genomic RNA, and BPL treatment does not affect viral infectivity (Desbat *et al.*, 2011; Budimir *et al.*, 2012), we chose this inactivation method. Inactivation of hPIV2ΔF/EGFP was confirmed by fluorescence microscopy and no cytopathic effects were observed (Fig. 4A and data not shown), Furthermore, no functional loss of envelope proteins was observed by HA assay (Fig. 4B). Figure 4C shows a comparison of DC maturation states induced by live hPIV2ΔF/EGFP and BPL-inactivated hPIV2ΔF/EGFP (BPL-hPIV2ΔF/EGFP).

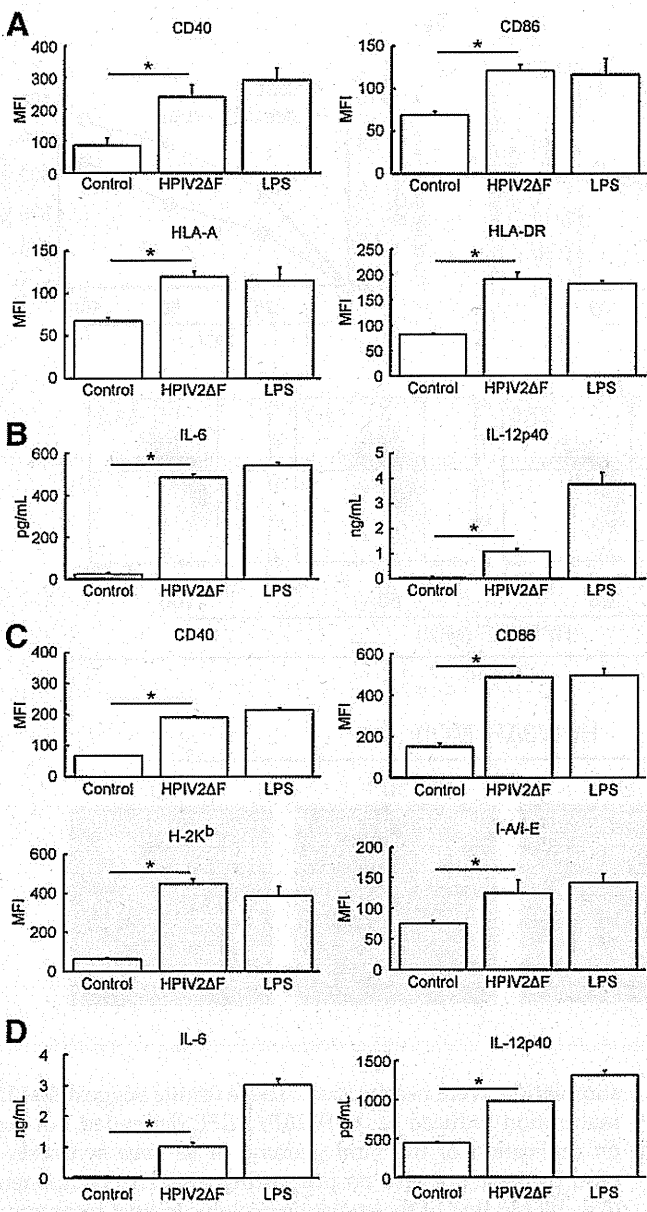

FIG. 3. HPIV2ΔF/EGFP-induced DC maturation and maturation state of DCs were comparable to those induced by LPS. MoDCs (**A** and **B**) and BMDCs (**C** and **D**) were unstimulated or stimulated either with LPS (1 μg/ml) or hPIV2ΔF/EGFP (MOI of 25) for 48 hr. The expression levels of maturation markers CD40, CD86, HLA-A (H-2K^b), and HLA-DR (I-A/I-E) were analyzed by flow cytometry. IL-6 and IL-12p40 in the supernatant were measured by ELISA. All data are shown as means ± SD, and statistical significance ($^*p < 0.05$) was determined by Bonferroni test.

Although BPL-hPIV2ΔF/EGFP exhibited significantly reduced DC-stimulatory activity compared with live hPIV2ΔF/EGFP, the expression levels of maturation markers on BMDCs stimulated with BPL-hPIV2ΔF/EGFP were significantly enhanced, compared with those on unstimulated BMDCs. Similar findings were also observed in cytokine release (Fig. 4D). These results suggest that preexisting hPIV2ΔF components per se have DC-stimulatory activity leading to DC maturation, albeit viral RNA synthesis may be required to induce DC maturation effectively.

Discussion

Previous epidemic data disclosed that about 60% of all hPIV2 infections occurred in children younger than 5 years old, and that the infection rate of hPIV2 was less frequent than that of other hPIVs (Henrickson, 2003). In addition, hPIV2 naturally reinfects healthy adults throughout life (Hall, 2001), which makes it likely that hPIV2-based vectors could be safely administered and escape from responses to the viral backbone. In the present study, we first investigated the transduction efficiency of hPIV2ΔF into human MoDCs. The results showed that hPIV2ΔF at an MOI of 25 more efficiently transduced the *EGFP* gene into MoDCs in comparison with a conventional retroviral (ReV) vector at 48 hr postinfection. Whereas the transduction efficiency of the ReV vector into nondividing cells, such as DCs, is generally low (Szabolcs *et al.*, 1997; Jenne *et al.*, 2001; Tan *et al.*, 2005), other commonly used vectors, such as an adenoviral vector or an adeno-associated viral vector, require an MOI greater than 100 to achieve sufficient transduction into DCs (Ponnazhagan *et al.*, 2001; Mercier *et al.*, 2003). Furthermore, the entire life cycle of hPIV2 takes place completely out of the nucleus, which excludes the risk of host genome alterations (Henrickson, 2003). Altogether, hPIV2ΔF could be useful as a novel cytoplasmic RNA viral vector to develop recombinant vaccines against various diseases.

Next, we compared the replication/transcription states of hPIV2ΔF in murine BMDCs and human MoDCs. Consistent with the previous reports (Ito *et al.*, 1989; Komada *et al.*, 2000), viral genomic copy numbers and viral transcripts of hPIV2ΔF in BMDCs were significantly lower than those in MoDCs. However, hPIV2ΔF/EGFP transduction of BMDCs showed MOI-dependent propagation, and about 70% of BMDCs expressed EGFP at an MOI of 100. Studies have revealed that the V protein of hPIV2 inhibited interferon (IFN) production and signaling by degrading human STAT2, but not murine STAT2 (Nishio *et al.*, 2001, 2005; Parisien *et al.*, 2001). Furthermore, V-knockout hPIV2 induced higher IFN production and viral replication was restricted in the human cell line (Schaap-Nutt *et al.*, 2010). Although IFN signaling was not investigated in our study, similar mechanisms may be involved in less propagation of hPIV2ΔF in primary murine cells, compared with human cells.

DC maturation is an essential event to trigger immunity. In presenting antigen to naive T cells, costimulatory molecule-related signals from DCs are important for the fate of naive T cells in addition to the signals through the MHC–T cell receptor (TCR) complex (Guermonprez *et al.*, 2002; de Jong *et al.*, 2005). Moreover, T cell polarization is controlled by a number of cytokines from DCs, and they reciprocally regulate the differentiation of naive T cells into each T cell subset (Watford *et al.*, 2003; Feili-Hariri *et al.*, 2005; Zhou *et al.*, 2009). This study demonstrated that hPIV2ΔF stimulation sufficiently increased the expression of MHCs and costimulatory molecules on MoDCs and BMDCs to a level comparable with LPS stimulation. IL-6 and IL-12p40 are proinflammatory cytokines that regulate T cell polarization (Egwuagu, 2009; Kimura and Kishimoto, 2010; Prochazkova *et al.*, 2012). Secretion of both IL-6 and IL-12p40 from MoDCs and BMDCs was significantly increased by hPIV2ΔF stimulation, suggesting functional maturation of DCs. Interestingly, despite the low replication/transcription of hPIV2ΔF/

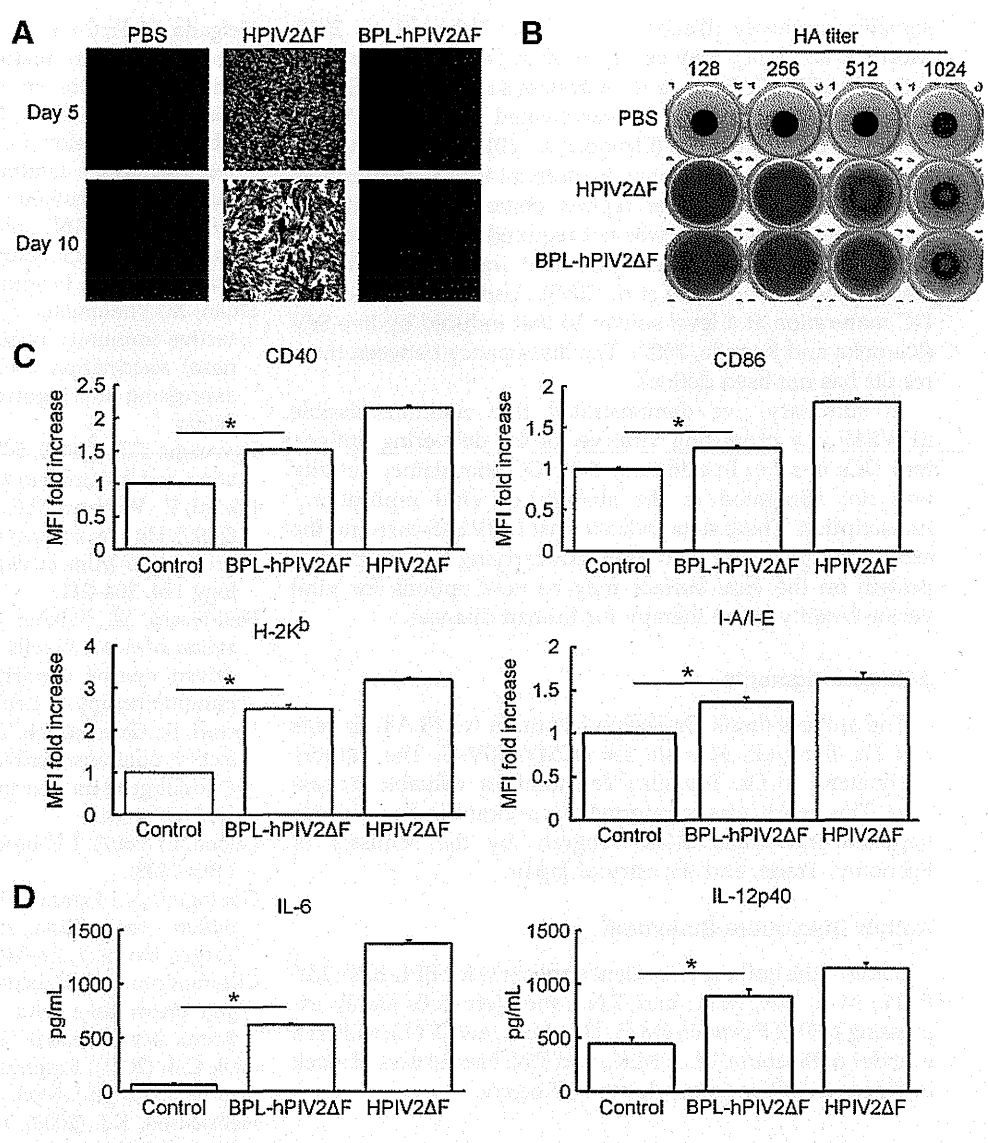

FIG. 4. Effects of β-propiolactone (BPL)-inactivated hPIV2ΔF/EGFP on DC maturation. BMDCs were unstimulated or stimulated with either hPIV2ΔF/EGFP or BPL-hPIV2ΔF/EGFP at an MOI of 25 for 48 hr. (A) Inactivation of hPIV2ΔF/EGFP by BPL was confirmed by the absence of EGFP expression as determined by fluorescence microscopy in the packaging cell line for hPIV2ΔF. (B) Effect of BPL on hPIV2ΔF/EGFP envelope was determined by HA assay. (C) The expression levels of maturation markers CD40, CD86, H-2K^b, and I-A/I-E were analyzed by flow cytometry. (D) IL-6 and IL-12p40 in the supernatant were measured by ELISA. All data are shown as means±SD, and statistical significance ($^*p<0.05$) was determined by Bonferroni test.

EGFP in BMDCs (Fig. 2A and B), the maturation state of BMDCs was nearly equal to that of MoDCs, in each comparison with LPS stimulation of each cell type, implying that DC maturation was triggered by preexisting hPIV2ΔF components without viral replication/transcription events.

Finally, we genomically inactivated hPIV2ΔF by BPL treatment (BPL-hPIV2ΔF) to investigate whether DC maturation was induced by preexisting hPIV2ΔF components alone. BPL completely inactivated hPIV2ΔF replication/transcription without affecting viral envelope function, suggesting that BPL is a useful viral inactivator in developing safer vaccines. Although the DC-stimulatory activity of the BPL-hPIV2ΔF vehicle itself was attenuated in comparison with live hPIV2ΔF, BPL-hPIV2ΔF still possessed DC-stimulatory activity without viral replication/transcription. In fact, hPIV2ΔF inactivated with BPL at a lower concentration (0.012%, v/v), despite the lack of viral replication/transcription, markedly enhanced its DC-stimulatory activity compared with virus inactivated with 0.05% BPL (data not shown). These findings suggest that hPIV2ΔF, the adjuvanticity of which is still maintained after genomic inactivation, would be useful as a safe vector for recombinant vaccines, and the strength of BPL inactivation might influence viral adjuvanticity, probably by affecting intraviral structure.

Viral inactivation with chemical reagents (e.g., formalin) or physical treatments (e.g., heat and ultraviolet [UV]) is likely desirable for developing safe vaccines, and some inactivated vaccines are currently used. However, vaccine efficacy is still controversial from immunological points of view (Delgado *et al.*, 2009).

In DC monitoring of viral infection, two major sensors exist. One is the family of Toll-like receptors (TLRs), and the other is the family of cytosolic RNA helicases such as retinoic acid-inducible gene I (RIG-I) or melanoma differentiation-associated gene 5 (MDA5) (Meylan *et al.*, 2006; Pichlmair *et al.*, 2006; Barral *et al.*, 2009). In these sensors, murine myeloid DCs detect viral infection by recognizing uncapped 5′-triphosphates of viral single-stranded RNA (ssRNA) or double-stranded RNA (dsRNA) through cytosolic RNA helicases (Kato *et al.*, 2005). Our finding predicts that hPIV2ΔF-induced DC maturation occurs in the absence of viral replication/transcription, suggesting that alternative viral sensors, for example, a TLR- or autophagy-mediated

signaling pathway (Bieback *et al.*, 2002; Shirey *et al.*, 2010; Morris *et al.*, 2011), may be involved in DC maturation subsequent to hPIV2ΔF infection. A previous report using Sendai virus (SeV) showed that UV-inactivated SeV (UV-SeV) induced no DC maturation (Okano *et al.*, 2011), suggesting that viral replication/transcription is essential for DC maturation. On the other hand, other reports demonstrated that SeV replication/transcription was not required for the expression of DC maturation markers, whereas it was required for cytokine production (López *et al.*, 2003). Also, UV-SeV induced DC maturation at a level similar to that induced by live SeV (Kurooka and Kaneda, 2007). The discrepancy between these results has not been defined.

In summary, we demonstrated that nontransmissible hPIV2ΔF is a promising viral vector for delivering antigen into DCs *in vitro*. In addition, their DC-stimulatory activity was not abrogated in the absence of viral replication/transcription. These data indicate that hPIV2ΔF carrying the exogenous gene and BPL-hPIV2ΔF carrying the exogenous protein on the viral surface may be new options for viral vector-based vaccine therapy for human diseases.

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Author Disclosure Statement

Some of the authors are patent applicants for BPL-hPIV2ΔF (K.H., M.F., J.O., M.K., and T.N.) and Vero cells stably expressing hPIV2 F protein (M.F., J.O., M.K., and T.N.). M.F. is a founder of Biocomo. M.F., M.K., and T.N. have shares of stock in Biocomo. J.O. is an employee of Biocomo.

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原　著

小児におけるインフルエンザHAワクチン接種量変更による効果と安全性の検討

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要　旨

小児におけるインフルエンザHAワクチンの年齢別1回接種量の規定を見直す基礎資料を得る目的で，WHO推奨用量と同一の年齢別接種量に増量した場合の有効性，安全性を検討した．国内で承認されているワクチンとして阪大微生物病研究会製，対照群としてサノフィパスツール社製を用い，6カ月～13歳未満児を対象に0.25mL接種群と0.5mL接種群を設定し，罹患状況，副反応発現状況等を調査し，接種前，2回接種後のHI抗体価を測定した．また，HI抗体価の結果を補完するため中和抗体価を測定した．その結果，A/H1N1型のHI抗体価は両接種群でビケン製，サノフィ製ともに良好な上昇傾向を示し，中和抗体価も同傾向を示した．A/H3N2型のHI抗体価はビケン製では全体に低値であったが，中和抗体価はビケン製，サノフィ製とも良好な上昇傾向を示した．B型のHI抗体価はビケン製，サノフィ製とも顕著な上昇傾向を示さなかったが，中和抗体価はともに良好な上昇傾向を示した．当該シーズンのインフルエンザ流行がA/H1N1型のほぼ単独流行であったことからA/H1N1型について発症に関する要因解析を行ったところ，発症リスクを下げる要因として接種後HI抗体価が40倍以上に上昇していることが有意となった．また，接種後HI抗体価40倍以上上昇群に対する同20倍以下群の発熱に関する相対危険が有意に高く，抗体価の高い群で発熱の程度が抑制される傾向がみられたことから，本研究の接種量で用いた両ワクチンとも発症予防効果を有すると認められ，さらに接種時に重篤な副反応の発現を認めず，同等の安全性を有すると考えられた．以上のことから，小児に対してインフルエンザHAワクチンをWHO推奨用量で接種した際の有効性，安全性を確認することができた．

〔感染症誌　87：195～206，2013〕

序　文

わが国におけるインフルエンザHAワクチンの小児における接種量は，2011/12シーズンからWHO推奨用量（6カ月～3歳未満：0.25mL，3歳以上：0.5mL）[1]に増量されたところであるが，それ以前は科学的根拠も不明確なまま，全粒子ワクチンの接種規定（6カ月～1歳未満：0.1mL，1～6歳未満：0.2mL，6～13歳未満：0.3mL，13歳以上：0.5mL）が適用されていた．わが国のワクチンは小児に対して効果が低いとされていたが，欧米のワクチンは小児に対しても効果があるとされ，ワクチンの質および接種量の違いが原因と考えられていた[2]．加えて，細分化された接種規定では新型インフルエンザのパンデミック発生時に混乱の元となることも危惧されていた．そこで，WHO推

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奨用量によるワクチンの有効性と安全性の評価を行うことを目的として，2007/08シーズンに三重県内11カ所の調査対象施設の小児科を受診した6カ月～13歳未満の小児を対象に，欧米諸国と同じ接種量である0.25mL接種群，0.5mL接種群に分け，わが国の現行のインフルエンザHAワクチンとして一般財団法人阪大微生物病研究会製フルービックHA（以下；ビケン製），対照群としてサノフィパスツール社製VAXIGRIP（以下；サノフィ製）のワクチンを用い，接種によるHI抗体価上昇と副反応発現の状況を比較し，ワクチン効果と安全性について検討した．HI抗体価の結果を補完するため，中和抗体価を測定した．また，サノフィ製ワクチンの日本人小児での有効性・安全性についても検討した．

対象と方法

1. 調査対象者

三重県内の小児科11カ所（1カ所：桑名市，四日市市，亀山市，松阪市，伊勢市，鳥羽市，名張市，2カ所：鈴鹿市，津市）を対象に，医療機関ごとに受診した小児の保護者のインフォームドコンセントを得た上で，6カ月～3歳未満児300人，3歳～13歳未満児300人を目標に協力者を募り，協力が得られた6カ月～3歳未満児253人，3歳～13歳未満児307人，合計560人を調査対象とした．

2. ワクチン接種

使用したワクチンは，ビケン製はA/Solomon lslands/3/2006 (H1N1) 15.6μg，A/Hiroshima/52/2005 (H3N2) 15.3μg，B/Malaysia/2506/2004 (B) 16.1μg，サノフィ製はA/Solomon lslands/3/2006 IVR-145 (H1N1)，A/Wisconsin/67/2005 NYMC X-161B (H3N2)，B/Malaysia/2506/2004 like strains (B) 各15μgを含む不活化スプリットワクチンであった．接種方法は，サノフィ製の添付文書に沿い，6カ月～3歳未満児に対しては0.25mL，3歳～13歳未満児に対しては0.5mLを4週間隔で2回皮下注射した．なお，サノフィ製はわが国では承認されていないため研究者が個人輸入して使用した．

3. 調査情報

対象者の属性は，保護者記入用調査票により性，生年月日，体重，通園状況，家族状況，過去3シーズンの接種歴，前シーズン（2006/07シーズン）のインフルエンザ罹患歴を，医療機関記入用調査票により当該シーズン（2007/08シーズン）の接種年月日，採血年月日，基礎疾患，受診時のインフルエンザ迅速診断キット使用状況および臨床症状等の情報を得た．また，接種後の副反応（接種後48時間以内の全身副反応と局所副反応）および調査期間中（2007年12月17日～2008年3月30日）のインフルエンザ罹患状況は，調査票返信葉書により情報を得た．

4. HI抗体測定

1）採血時期

対象者（ビケン製接種群，サノフィ製接種群）の採血は，接種前と2回接種後（2回目接種から4週間後）に実施した．

2）HA抗原

ビケン製接種群には2007/08シーズンワクチン株（デンカ生研）を用い，サノフィ製接種群にはサノフィパスツール社から提供された2007/08シーズンWHO推奨ワクチン株を用いた．なお，HA抗原同士の凝集塊形成を除去する目的で超音波処理を行った後に使用した．

3）赤血球凝集抑制（HI）抗体測定

RDE処理後，10倍から2倍階段希釈した血清25μLにHA抗原4HA単位/25μLおよび0.5%ニワトリ赤血球50μLを添加するWHO方式により行い，HIを示した血清の最大希釈倍数を抗体価とした．この測定は三重県保健環境研究所で実施した．

5. 中和抗体測定

マイクロ中和試験法に準じて実施した[3]．RDE処理後，10倍から4倍段階希釈した血清30μLに至適濃度に調製したウイルス（2007/08シーズンインフルエンザHAワクチン株）を等量添加し，96穴プレートにて37℃，60分中和反応を行い，あらかじめ前日に96穴平底プレートに培養しておいたMDCK細胞に中和反応液30μLを添加し，CO_2インキュベータ内で37℃，16～24時間培養した．培養後，エタノールにて感染細胞を固定し，PAP染色を行い，染色された細胞（フォーカス）の数を鏡顕下でカウントし，ウイルスコントロールの数の50%のフォーカス数を基準に中和抗体価を算出するPAP法にて測定した．この測定は阪大微生物病研究会で実施した．

6. 解析方法

1）インフルエンザ発症に関連する要因

インフルエンザ発症に関連する要因を検討するため，当該シーズンのインフルエンザ発症を目的変数に，ワクチン種別，性別，過去3年間の予防接種歴の有無，前シーズンA型インフルエンザ発症の有無，前シーズンB型インフルエンザ発症の有無，ワクチン接種量，アレルギー（喘息又はアトピー）の有無，通園状況，兄弟姉妹の有無，2回接種後HI抗体価について感染防御水準[4)~8)]とされている40倍以上のHI抗体価の有無の12項目を説明変数として多重ロジスティックモデルによる解析を行った．分析に際し，調査参加者560人から1回目と2回目の接種量が異なる7人，毎週の臨床症状情報に欠落のあった3人，2回目の接種時期が臨床症状調査を開始した2007年第51週以降

Table 1 Stydy subjects broken down by age and the vaccine administered

	age														total
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	
male	12	68	53	25	36	31	23	18	15	7	11	4	5	1	309
female	14	61	45	19	29	13	21	8	11	14	9	5	2		251
BIKEN	13	65	49	24	37	23	24	13	12	7	10	6	2		285
Sanofi Pasteur	13	64	49	20	28	21	20	13	14	14	10	3	5	1	275
total	26	129	98	44	65	44	44	26	26	21	20	9	7	1	560

となった50人を除く500人のうち，前記12項目の情報に欠落がない480人を対象とした．また，接種前のA/H1N1型に対するHI抗体価が10倍以下の者を対象とした分析は，480人から接種前HI抗体価20倍以上の96人を除く384人を対象とした．

2) ワクチン接種前後のHI抗体価推移の解析

ワクチン種別，接種量別に接種前と2回接種後でのHI抗体価の推移をウイルス型ごとにまとめるとともに，欧州医薬品庁（EMEA）の基準[9]に基づく幾何平均抗体価変化率（Geometric mean fold rise以下GMFR），抗体陽転率（Seroconversion rate以下SCR）を求め，比較した．HI抗体価の結果を補完するため中和抗体価を測定し，GMFRおよびHI抗体価に対するSpearmanの順序相関係数を算出した．さらに，接種によるHI抗体上昇に影響する要因を検討するため，接種前HI抗体価が10倍以下の者を対象として，接種後にHI抗体価が40倍以上に上昇したことを目的変数に，ワクチン種別，接種量別，性別，過去3年間の接種歴の有無，前シーズンA型インフルエンザ発症の有無，前シーズンB型インフルエンザ発症の有無，アレルギー（喘息またはアトピー）の有無の7項目を説明変数として多重ロジスティックモデルによる解析を行った．分析に際しては，500人のうち前記7項目の情報に欠落がないことに加えて，接種前抗体価が10倍以下の者と，A/H1N1型については385人，A/H3N2型は287人，B型は482人を対象とした．

3) ワクチン接種後の副反応発現状況の解析

1回目接種後および2回目接種後の48時間以内の副反応について，全身副反応として37.5℃以上の発熱，発疹，局所副反応として接種部位の発赤，腫脹，硬結の発現率をワクチン種別，接種量別に比較した．

7. 倫理審査

本研究は，2007年10月16日に開催の国立病院機構三重病院倫理審査委員会において承認を得た（受付番号19-22，題名「小児におけるインフルエンザHAワクチンの接種量と効果に関する研究」）．

結　果

1. 調査対象者

県内のほぼ全域から，6カ月～3歳未満児253人，3歳～13歳未満児307人（13歳1人を含む）の合計560人の協力が得られ，性別内訳は男309人，女251人であった．年齢別内訳をTable 1に示した．

2. ワクチン接種状況

対象者には1回目と2回目の接種量が異なるものが含まれており，0.25mLを2回接種したものはビケン製126人，サノフィ製125人，0.50mLを2回接種したものはビケン製155人，サノフィ製147人，合計553人であった．なお，接種時期については，1回目は2007年第42週から第48週，2回目は2007年第46週から2008年第4週であり，2回目の接種が臨床症状の調査開始週（第51週）以降となったのは調査対象者560人中50人であった．

3. 調査対象者のインフルエンザ発症状況

三重県における感染症発生動向調査の結果，2007/08シーズンのインフルエンザの流行は，2008年第4週をピークとするA/H1N1型のほぼ単独流行であった．各調査対象機関でインフルエンザと診断された人数は調査対象者560人中47人（8.4%）で，診断週は2008年第4週から第6週に集中しており，感染症発生動向調査と同様の傾向であった．なお，罹患者47人の内訳は，ビケン製0.25mL接種者11人，同0.50mL接種者14人，サノフィ製0.25mL接種者8人，同0.50mL接種者14人であった．

4. 解析

1) インフルエンザ発症に関連する要因

2007/08シーズンのインフルエンザ発症に関連する要因について多重ロジスティックモデルによる解析を行ったところ，発症リスクを高める要因として有意となったのは「2回接種後のA/H1N1型に対するHI抗体価が20倍以下」（オッズ比4.10，p＝0.0004）のみであった．なお，HI抗体保有に係る調査年の流行による影響を避けるため，解析に際しては2007年第51週の前に2回接種が完了した者を解析対象とした（Table 2）．

Table 2 Factor analysis for influenza contraction by the univariate analysis (χ^2 test) and the multivariate (multiple logistic model) analysis

Qualitative explanatory variable	Category	Number of occurrence / Number of subjects*1	χ^2 test RRs*2	χ^2 test p value	Multiple logistic model ORs*3	Multiple logistic model (95% CI)	Multiple logistic model p value
(1) Vaccine	BIKEN	18 / 242	1		1		
	Sanofi Pasteur	19 / 238	1.07	0.958	1.02	(0.48-2.17)	0.951
(2) Sex	Male	18 / 268	1		1		
	Female	19 / 212	1.33	0.457	1.35	(0.67-2.71)	0.396
(3) Vaccination record for the past 3years	−	8 / 148	1		1		
	+	29 / 332	1.62	0.281	1.35	(0.48-3.78)	0.568
(4) Last winter type A patients	−	36 / 439	1		1		
	+	1 / 41	0.30	0.309	0.33	(0.04-2.60)	0.292
(5) Last winter type B patients	−	33 / 449	1		1		
	+	4 / 31	1.76	0.439	2.39	(0.70-8.22)	0.166
(6) Vaccinated dose (age)	0.25mL (<3 years)	15 / 214	1		1		
	0.50mL (≧3 years)	22 / 266	1.18	0.732	1.66	(0.57-4.84)	0.352
(7) Asthma or atopic dermatitis	−	31 / 419	1		1		
	+	6 / 61	1.33	0.682	1.31	(0.49-3.48)	0.595
(8) Preschool	−	14 / 164	1		1		
	+	23 / 316	0.85	0.757	0.47	(0.17-1.33)	0.157
(9) Siblings	−	4 / 50	1		1		
	+	33 / 430	0.96	1.000	0.98	(0.30-3.15)	0.970
(10) HI Antibody titer after the vaccination A/H1N1	≧40-fold	17 / 354	1		1		
	≦20-fold	20 / 126	3.31	0.0001	4.10	(1.87-9.00)	0.0004***
(11) HI Antibody titer after the vaccination A/H3N2	≧40-fold	25 / 324	1		1		
	≦20-fold	12 / 156	1.00	1.000	0.65	(0.27-1.57)	0.338
(12) HI Antibody titer after the vaccination B	≧40-fold	6 / 106	1		1		
	≦20-fold	31 / 374	1.46	0.491	1.23	(0.46-3.29)	0.681

*1: Number of analysis subjects 480
*2: RR is risk ratio (Ratio of incidence)
*3: OR is adjusted odds ratio by multiple logistic model
Annotation *2 and *3 are the same in Table 3, 6, 7 and 8
***p<0.001

この結果から，さらに解析対象を接種前のHI抗体価が10倍以下の者とし，説明変数のうちHI抗体価はA/H1N1型のみとして解析を行ったところ，発症リスクを高める要因として有意となったのは「2回接種後のA/H1N1型に対するHI抗体価が20倍以下（オッズ比3.12，p=0.003）」，「前シーズンB型インフルエンザ発症（オッズ比3.85，p=0.043）」であった（Table 3）．

2）抗体価

抗体価の解析は，2007/08シーズンのインフルエンザ流行がA/H1N1型のほぼ単独流行であったことから，この影響を避けるため，A/H1N1型については2007年第51週の前に2回接種が完了した者を対象として行った．A/H1N1型のHI抗体価は0.25mL接種群，0.50mL接種群でビケン製，サノフィ製ともに欧州医薬品庁（EMEA）の基準を満たす良好な上昇傾向（0.25mL接種群ビケン製：GMFR 6.8，SCR 60.7%，サノフィ製：GMFR 13.4，SCR 76.6%，0.50mL接種群ビケン製：GMFR 7.0，SCR 68.6%，サノフィ製：GMFR 5.1，SCR 60.9%）を示し，中和抗体価も同様の傾向（0.25mL接種群ビケン製：GMFR 30.4，サノフィ製：GMFR 57.2，0.50mL接種群ビケン製：GMFR 14.6，サノフィ製：GMFR 11.0）を示した．A/H3N2型のHI抗体価は0.25mL接種群，0.50mL接種群とも，ビケン製に比べサノフィ製が良好な上昇傾向（0.25mL接種群ビケン製：GMFR 2.3，SCR 23.0%，サノフィ製：GMFR 8.4，SCR 73.9%，0.50mL接種群ビケン製：GMFR 1.9，SCR 16.8%，サノフィ製：GMFR 2.4，SCR 31.3%）を示したが，中和抗体価はこれと異なり，ビケン製，サノフィ製とも良好な上昇傾向（0.25mL接種群ビケン製：GMFR 23.2，サノフィ製：GMFR 16.0，0.50mL接種群ビケン製：GMFR 2.7，サノフィ製：GMFR 1.9）を示した．B型では，両接種群でビケン製，サノフィ製ともに顕著な上昇傾向は認められなかった（0.25mL接種群ビケン製：GMFR 2.2，SCR 16.7%，サノフィ製：GMFR 2.0，SCR 10.4%，0.50mL

Table 3 Factor analysis for influenza contraction of subjects with an antibody titer of 10-fold or less before vaccination by the univariate analysis (χ^2 test) and the multivariate (multiple logistic model) analysis

Qualitative explanatory variable	Category	Number of occurrence / Number of subjects*1	χ^2 test		Multiple logistic model		
			RR*2	p value	OR*3	(95% CI)	p value
(1) Vaccine	BIKEN	16 / 194	1		1		
	Sanofi Pasteur	19 / 190	1.21	0.675	1.37	(0.66-2.87)	0.399
(2) Sex	Male	17 / 211	1		1		
	Female	18 / 173	1.29	0.537	1.38	(0.67-2.85)	0.377
(3) Vaccination record for the past 3 years	−	8 / 134	1		1		
	+	27 / 250	1.81	0.167	1.39	(0.49-3.94)	0.537
(4) Last winter type A patients	−	34 / 357	1		1		
	+	1 / 27	0.39	0.505	0.41	(0.05-3.37)	0.404
(5) Last winter type B patients	−	31 / 367	1		1		
	+	4 / 17	2.79	0.093	3.85	(1.04-14.24)	0.043*
(6) Vaccinated dose (age)	0.25mL (<3 years)	15 / 203	1		1		
	0.50mL (≧3 years)	20 / 181	1.50	0.286	1.79	(0.61-5.27)	0.287
(7) Asthma or atopic dermatitis	−	30 / 340	1		1		
	+	5 / 44	1.29	0.785	1.23	(0.42-3.61)	0.703
(8) Preschool	−	14 / 157	1		1		
	+	21 / 227	1.04	0.945	0.51	(0.18-1.43)	0.201
(9) Siblings	−	4 / 44	1		1		
	+	31 / 340	1.00	1.000	0.89	(0.27-2.92)	0.846
(10) HI Antibody titer after the vaccination A/H1N1	≧40-fold	15 / 259	1		1		
	≦20-fold	20 / 125	2.76	0.002	3.12	(1.46-6.69)	0.003**

*1: Number of analysis subjects 384
*p<0.05
**p<0.01

接種群ビケン製：GMFR 2.0，SCR 17.4%，サノフィ製：GMFR 1.8，SCR 12.4%）が，中和抗体価は，A/H1N1型，A/H3N2型に比較するとやや低いものの，ともに良好な上昇傾向（0.25mL接種群ビケン製：GMFR 14.4，サノフィ製：GMFR 11.4，0.50mL接種群ビケン製：GMFR 4.3，サノフィ製：GMFR 4.3）を示した（Table 4，5）．なお，HI抗体価と中和抗体価は良好な相関（Spearmanの順序相関係数：0.711～0.915）を示した（Table 4）．

次に，2回接種後にHI抗体価が40倍以上に上昇したことと関連する要因について多重ロジスティックモデルにより解析した．A/H1N1型では，抗体上昇にプラスに影響する要因として有意となったのは「サノフィ製ワクチンを接種したこと（オッズ比1.72，p=0.019）」，「前シーズンA型インフルエンザ発症（オッズ比2.93，p=0.044）」，「接種量0.50mL（オッズ比2.12，p=0.007）」であり，抗体上昇にマイナスに影響する要因として有意となったのは「過去3年間にワクチン接種歴があること（オッズ比0.25，p=0.000）」，「アレルギーがあること（オッズ比0.44，p=0.016）」であった（Table 6）．A/H3N2型では，抗体上昇にプラスに影響する因子として「サノフィ製ワクチンを接種すること（オッズ比10.07，p=0.000）」のみで有意となった（Table 7）．B型では，抗体上昇にプラスに影響する要因として「前シーズンB型インフルエンザ発症（オッズ比3.75，p=0.006）」のみで有意となった（Table 8）．

さらに，多重ロジスティックモデルによる分析の結果，発症リスクを下げる要因として「2回接種後のA/H1N1型に対するHI抗体価が40倍以上」が有意となったことから，2007年第50週以前に2回のワクチン接種が完了している500人について，A/H1N1型に対する2回接種後のHI抗体価レベル別，発症時の最高発熱レベル別発熱者数，発熱率をまとめた（Table 9）．「2回接種後のA/H1N1型に対するHI抗体価が40倍以上」を指標に38.5℃以上の発熱に対する相対危険（RR）を算出したところ，40倍以上群に対する20倍以下群のRRは2.9（p=0.0010）と有意に高く，「2回接種後のA/H1N1型に対するHI抗体価が80倍以上」を指標とすると，その傾向はより強くなった（RR=3.5，p=0.0007）．

3）ワクチン接種後の副反応発現状況

1回目接種後および2回目接種後の副反応について，ワクチン種別，接種量別の37.5℃以上の発熱，発疹，発赤，腫脹，硬結，疼痛の発現数および発現率（%）を取りまとめたところ，重篤な副反応は1回目，2回

Table 4 Change of geometric mean in the antibody titer

Dose	Method	Vaccine	A/H1N1 mean antibody titer						A/H3N2 mean antibody titer						B mean antibody titer					
			Subjects*1	Before vaccination	R*2	After the second vaccination	R	GMFR*3	Subjects	Before vaccination	R	After the second vaccination	R	GMFR	Subjects	Before vaccination	R	After the second vaccination	R	GMFR
0.25mL (<3 years)	HI test	BIKEN	107	6.0	0.711	40.8	0.830	6.8	126	7.6	0.836	17.1	0.817	2.3	126	5.1	0.734	11.4	0.842	2.2
		Sanofi Pasteur	111	6.1	0.722	82.0	0.868	13.4	125	11.1	0.915	93.4	0.814	8.4	125	5.2	0.730	10.2	0.835	2.0
	NT test	BIKEN	106	7.3	0.711	221.9	0.830	30.4	125	40.9	0.836	948.8	0.817	23.2	125	7.5	0.734	107.9	0.842	14.4
		Sanofi Pasteur	111	8.4	0.722	480.2	0.868	57.2	125	63.4	0.915	1,014.1	0.814	16.0	125	8.1	0.730	92.4	0.835	11.4
0.50mL (≧3 years)	HI test	BIKEN	140	11.4		79.6		7.0	155	19.4		36.4		1.9	155	7.7		15.4		2.0
		Sanofi Pasteur	135	13.3		68.2		5.1	144	75.5		177.9		2.4	144	8.0		14.6		1.8
	NT test	BIKEN	138	26.5		387.3		14.6	153	1,388.8		3,762.5		2.7	153	44.4		190.9		4.3
		Sanofi Pasteur	136	46.6		511.4		11.0	144	2,609.8		4,926.6		1.9	144	45.1		194.0		4.3
0.25mL (2 years)	HI test	BIKEN	45	6.7		43.9		6.6	48	8.3		22.8		2.7	48	5.1		14.6		2.9
		Sanofi Pasteur	44	6.9		55.7		8.1	49	18.4		85.9		4.7	49	5.4		11.4		2.1
	NT test	BIKEN	45	10.8		235.2		21.8	48	127.0		1,890.3		14.9	48	10.9		164.7		15.1
		Sanofi Pasteur	44	12.1		357.3		29.5	49	224.7		1,582.6		7.0	49	12.0		125.8		10.5
0.50mL (3 years)	HI test	BIKEN	20	6.8		49.2		7.2	23	13.5		26.2		1.9	23	5.5		11.6		2.1
		Sanofi Pasteur	20	6.8		42.9		6.3	20	26.4		117.1		4.4	20	5.2		10.0		1.9
	NT test	BIKEN	20	13.2		251.1		19.0	23	584.7		2,638.3		4.5	23	13.9		122.0		8.8
		Sanofi Pasteur	20	11.5		278.6		24.2	20	485.0		3,151.7		6.5	20	10.0		121.3		12.1

*1: Analysis of A/H1N1 mean antibody titer was perfomed in the subjects who received the second vaccination before 50th week in 2007 (non-epidemic periods of A/H1N1 subtype virus)

*2: Spearman rank correlation coefficient between HI antibody titer and NT antibody titer (0.25mL + 0.50mL)

*3: Geometric mean fold rise

Table 5 Seroconversion rate of the HI antibody titer

Dose	Vaccine	A/H1N1 HI antibody titer after the second vaccination					A/H3N2 HI antibody titer after the second vaccination					B HI antibody titer after the second vaccination				
		Subjects*1	NSC*2	SCR (%)*3	RR*4	p value	Subjects	NSC	SCR (%)	RR	p value	Subjects	NSC	SCR (%)	RR	p value
0.25mL (<3 years)	BIKEN	107	65	(60.7)	1		126	29	(23.0)	1		126	21	(16.7)	1	
	Sanofi Pasteur	111	85	(76.6)	1.26	0.012	119	88	(73.9)	3.21	0.000	125	13	(10.4)	0.62	0.147
0.50mL (≧3 years)	BIKEN	137	94	(68.6)	1		155	26	(16.8)	1		155	27	(17.4)	1	
	Sanofi Pasteur	133	81	(60.9)	0.89	0.185	128	40	(31.3)	1.86	0.004	145	18	(12.4)	0.71	0.225
0.25mL (2 years)	BIKEN	45	26	(57.8)	1		48	14	(29.2)	1		48	12	(25.0)	1	
	Sanofi Pasteur	44	27	(61.4)	1.06	0.730	46	26	(56.5)	1.94	0.007	49	6	(12.2)	0.49	0.106
0.50mL (3 years)	BIKEN	20	13	(65.0)	1		23	6	(26.1)	1		23	5	(21.7)	1	
	Sanofi Pasteur	20	13	(65.0)	1.00	1.000	17	11	(64.7)	2.48	0.015	20	3	(15.0)	0.69	0.571

*1: Analysis of A/H1N1 mean HI antibody titer was performed in the subjects who received the second vaccination before 50th week in 2007 (non-epidemic periods of A/H1N1 subtype virus)

*2: Number of seroconversions or significant increase (Standards by The European Agency for the Evaluation of Medicinal Products)

*3: Seroconversion rate

*4: Relative risk of "Sanofi Pasteur SCR" was set to 1 if the "BIKEN SCR"

Table 6 Factor analysis for acquisition of an A/H1N1 HI antibody titer of 40-fold or more after the second vaccination by the univariate analysis (χ^2 test) and the multivariate (multiple logistic model) analysis

Qualitative explanatory variable	Category	Number of occurrence / Number of subjects*1	χ^2 test		Multiple logistic model		
			RR*2	p value	OR*3	(95% CI)	p value
(1) Vaccine	BIKEN	122 / 195	1		1		
	Sanofi Pasteur	138 / 190	1.16	0.045	1.72	(1.09-2.70)	0.019*
(2) Sex	Male	140 / 212	1		1		
	Female	120 / 173	1.05	0.559	1.23	(0.78-1.93)	0.375
(3) Vaccination record for the past 3 years	−	107 / 134	1		1		
	+	153 / 251	0.76	0.000	0.25	(0.14-0.45)	0.000***
(4) Last winter type A patients	−	238 / 358	1		1		
	+	22 / 27	1.23	0.164	2.93	(1.03-8.34)	0.044*
(5) Last winter type B patients	−	248 / 368	1		1		
	+	12 / 17	1.05	0.992	0.87	(0.28-2.65)	0.801
(6) Vaccinated dose (age)	0.25mL (<3 years)	138 / 203	1		1		
	0.50mL (≧3 years)	122 / 182	0.99	0.929	2.12	(1.23-3.67)	0.007**
(7) Asthma or atopic dermatitis	−	238 / 341	1		1		
	+	22 / 44	0.72	0.014	0.44	(0.23-0.86)	0.016*

*1: Number of analysis subjects 385

目ともに認められなかった（Table 10, 11）. 37.5℃以上の発熱は，概して0.50mL接種者（3歳以上）に比べ，0.25mL接種者（3歳未満）で発現率が高かったが，0.25mL接種者についてワクチン種別にみると，ビケン製の1回目での発現が少なく，2回目での発現率はビケン製，サノフィ製でほとんど差がみられなかった. 局所反応は，発疹を除き，0.25mL接種者に比べ，0.50mL接種者で発現率が高く，1回目と2回目でほぼ同じ傾向がみられた.

考　察

本調査は，6カ月～13歳未満の小児を対象に，わが国のインフルエンザHAワクチンをWHO推奨用量に増量して使用した場合の有効性，安全性を検討することにより，わが国の接種規定を見直す基礎資料を得ることを目的として実施した.

接種後のHI抗体価の幾何平均値をもとに有効性をみると，A/H1N1型については，ビケン製，サノフィ製ともに良好な上昇傾向が認められた. A/H3N2型については，サノフィ製がビケン製を上回る傾向がみられたが，この一因として，ビケン製接種群のHI抗体測定に使用したHA抗原（A/Hiroshima/52/2005株）のインヒビター感受性に変異が生じている可能性が指摘されており[10]，本研究に用いたRDE処理によるインヒビターの除去が不十分となり，低値となった

平成25年3月20日

Table 7 Factor analysis for acquisition of an A/H3N2 HI antibody titer of 40-fold or more after the second vaccination by the univariate analysis (χ^2 test) and the multivariate (multiple logistic model) analysis

Qualitative explanatory variable	Category	Number of occurrence / Number of subjects*1	χ^2 test		Multiple logistic model		
			RR*2	p value	OR*3	(95% CI)	p value
(1) Vaccine	BIKEN	34 / 168	1		1		
	Sanofi Pasteur	85 / 119	3.53	0.000	10.07	(5.69-17.82)	0.000***
(2) Sex	Male	57 / 158	1		1		
	Female	62 / 129	1.33	0.054	1.72	(0.98-3.04)	0.061
(3) Vaccination record for the past 3 years	−	67 / 142	1		1		
	+	52 / 145	0.76	0.068	0.78	(0.42-1.46)	0.434
(4) Last winter type A patients	−	116 / 280	1		1		
	+	3 / 7	1.03	1.000	2.29	(0.41-12.72)	0.345
(5) Last winter type B patients	−	116 / 281	1		1		
	+	3 / 6	1.21	0.992	2.37	(0.36-15.74)	0.372
(6) Vaccinated dose (age)	0.25mL (<3 years)	96 / 203	1		1		
	0.50mL (≧3 years)	23 / 84	0.58	0.003	0.69	(0.33-1.43)	0.313
(7) Asthma or atopic dermatitis	−	108 / 252	1		1		
	+	11 / 35	0.73	0.270	0.65	(0.26-1.62)	0.360

*1: Number of analysis subjects 287

Table 8 Factor analysis for acquisition of a B HI antibody titer of 40-fold or more after the second vaccination by the univariate analysis (χ^2 test) and the multivariate (multiple logistic model) analysis

Qualitative explanatory variable	Category	Number of occurrence / Number of subjects*1	χ^2 test		Multiple logistic model		
			RR*2	p value	OR*3	(95% CI)	p value
(1) Vaccine	BIKEN	42 / 246	1		1		
	Sanofi Pasteur	29 / 236	0.72	0.176	0.65	(0.39-1.10)	0.108
(2) Sex	Male	41 / 268	1		1		
	Female	30 / 214	0.92	0.791	0.89	(0.53-1.50)	0.673
(3) Vaccination record for the past 3 years	−	25 / 169	1		1		
	+	46 / 313	0.99	1.000	0.88	(0.46-1.68)	0.699
(4) Last winter type A patients	−	65 / 445	1		1		
	+	6 / 37	1.11	0.981	1.02	(0.40-2.63)	0.963
(5) Last winter type B patients	−	63 / 460	1		1		
	+	8 / 22	2.66	0.009	3.75	(1.47-9.56)	0.006**
(6) Vaccinated dose (age)	0.25mL (<3 years)	33 / 241	1		1		
	0.50mL (≧3 years)	38 / 241	1.15	0.607	1.12	(0.59-2.12)	0.721
(7) Asthma or atopic dermatitis	−	61 / 419	1		1		
	+	10 / 63	1.09	0.933	1.02	(0.48-2.16)	0.955

*1: Number of analysis subjects 482

可能性が考えられる．このことは，中和抗体価ではビケン製，サノフィ製ともに良好な上昇傾向を示したことからも窺えた．B型については両接種群でビケン製，サノフィ製ともに顕著なHI抗体価の上昇傾向は認められなかったが，前シーズンの主流株がワクチン株と同じVictoria系統であり，多重ロジスティックモデルによる解析から前シーズンに罹患歴のある児でのブースター効果を認め，ワクチン接種の有効性が示された．また，2007/08シーズンのインフルエンザ発症リスクを高める要因として，多重ロジスティックモデルによる解析から2回目接種後のA/H1N1型に対するHI抗体価が20倍以下であることが有意となった．併せて，2回接種後のHI抗体価が40倍以上上昇群に対する同20倍以下群の発熱に関する相対危険が有意に高く，抗体価の高い群で発熱の程度が抑制される傾向がみられた．このことは，ワクチンメーカーの違いにかかわらず，今までの文献と同様に，HI抗体価40倍以上の保有が発症予防効果を示すことを支持する結果であった．一方で，40倍以上のHI抗体価上昇が認められたにもかかわらず，38.5℃以上の発熱を呈した

Table 9 Highest fever at the time of influenza contraction according to the A/H1N1 HI antibody titer after the second vaccination

Highest fever at the time of influenza contraction	A/H1N1 HI antibody titer after the second vaccination									Total
	≧1,280	640	320	160	80	40	20	10	<10	
Number of subjects in whom no fever developed	17	22	49	80	90	95	55	40	12	460
37.0 ~ 37.9℃					1					1
38.0 ~ 38.4℃							2	1		3
38.5 ~ 38.9℃			1	4		4	3		2	14
39.0 ~ 39.4℃			1		2	4	1	2	3	13
39.5 ~ 39.9℃					1	1	1	4		7
≧40℃							1		1	2
Number of patients 39.0℃ or more	0	0	1	0	3	5	3	6	4	22
(%)	(0.0)	(0.0)	(2.0)	(0.0)	(3.2)	(4.8)	(4.8)	(12.8)	(22.2)	(4.4)
Number of patients 38.5℃ or more	0	0	2	4	3	9	6	6	6	36
(%)	(0.0)	(0.0)	(3.9)	(4.8)	(3.2)	(8.7)	(9.5)	(12.8)	(33.3)	(7.2)
Number of patients 37.0℃ or more	0	0	2	4	4	9	8	7	6	40
(%)	(0.0)	(0.0)	(3.9)	(4.8)	(4.3)	(8.7)	(12.7)	(14.9)	(33.3)	(8.0)
Total	17	22	51	84	94	104	63	47	18	500

1: The square indicates those patients who ran a fever of 38.5℃ or more despite having an A/H1N1 antibody titer of 40-fold or greater.
Analysis of A/H1N1 was performed in the subjects who received the second vaccination before the 50th week in 2007.
2: The relative risk was 2.9 with a p value of 0.0010 with the Yates corrected chi-square analysis, representing a significant probability of generation of a fever of 38.5℃ or more in those subjects whose A/H1N1 HI antibody titer was less than 40-fold.
3: The relative risk was 3.5 with a p value of 0.0007 with the Yates corrected chi-square analysis, representing a significant probability of generation of a fever of 38.5℃ or more in those subjects whose A/H1N1 HI antibody titer was less than 80-fold.

Table 10 Adverse reactions after the first vaccination

Dose	Vaccine	Adverse reactions (Number of generation/Nnumber of answers) (%)					
		Fever ≧37.5℃	Rash	Redness	Swelling	Induration	Pain
0.25mL (<3 years)	BIKEN	1/126	8/127	11/127	3/127	12/127	1/127
	(%)	(0.8)	(6.3)	(8.7)	(2.4)	(9.4)	(0.8)
	Sanofi Pasteur	6/125	1/125	6/125	5/125	7/125	3/124
	(%)	(4.8)	(0.8)	(4.8)	(4.0)	(5.6)	(2.4)
	Subtotal	7/251	9/252	17/252	8/252	19/252	4/251
	(%)	(2.8)	(3.6)	(6.7)	(3.2)	(7.5)	(1.6)
0.50mL (≧3 years)	BIKEN	3/157	2/158	21/158	23/158	21/158	29/158
	(%)	(1.9)	(1.3)	(13.3)	(14.6)	(13.3)	(18.4)
	Sanofi Pasteur	5/147	0/149	23/149	36/149	22/149	41/149
	(%)	(3.4)	(0.0)	(15.4)	(24.2)	(14.8)	(27.5)
	Subtotal	8/304	2/307	44/307	59/307	43/307	70/307
	(%)	(2.6)	(0.7)	(14.3)	(19.2)	(14.0)	(22.8)
Total		15/555	11/559	61/559	67/559	62/559	74/558
(%)		(2.7)	(2.0)	(10.9)	(12.0)	(11.1)	(13.3)

No answer and unknown answers are excluded

者がみられたことにも留意する必要はある[8]. なお, 当該シーズンがA/H1N1型の単独流行であったことから, A/H1N1型に対する発症リスクに関する臨床効果は確認したが, A/H3N2型, B型に対する効果は検証できていないことを付記する.

副反応については, 発熱の発現率はビケン製がサノフィ製よりやや低く, 腫脹, 硬結, 疼痛はビケン製, サノフィ製とも0.50mL接種群でやや高めであったが, いずれも重篤なものではなく, 同程度の安全性が認められた.

また, 接種量について, 著者らが過去6シーズン(1999/2000~2004/05)に従来の用法・用量により実施した調査で, 0歳児に対する接種量0.1mLが十分でない可能性を指摘してきた[11)~14)]. 伊藤らはWHO推奨用量による(H1N1) 2009pdmワクチンの免疫原性について既承認用量接種群と比較した同一試験内におけ

Table 11 Adverse reactions after the second vaccination

Dose	Vaccine	Adverse reactions (Number of generation/Nnumber of answers) (%)					
		Fever 37.5℃ or more	Rash	Redness	Swelling	Induration	Pain
0.25mL (<3 years)	BIKEN	8/128	5/128	9/128	5/128	5/127	1/128
	(%)	(6.3)	(3.9)	(7.0)	(3.9)	(3.9)	(0.8)
	Sanofi Pasteur	8/125	2/124	7/125	5/125	4/125	6/125
	(%)	(6.4)	(1.6)	(5.6)	(4.0)	(3.2)	(4.8)
	Subtotal	16/253	7/252	16/253	10/253	9/252	7/253
	(%)	(6.3)	(2.8)	(6.3)	(4.0)	(3.6)	(2.8)
0.50mL (≧3 years)	BIKEN	4/155	2/156	17/156	21/156	22/154	37/156
	(%)	(2.6)	(1.3)	(10.9)	(13.5)	(14.3)	(23.7)
	Sanofi Pasteur	2/145	1/146	18/145	26/146	24/146	34/144
	(%)	(1.4)	(0.7)	(12.4)	(17.8)	(16.4)	(23.6)
	Subtotal	6/300	3/302	35/301	47/302	46/300	71/300
	(%)	(2.0)	(1.0)	(11.6)	(15.6)	(15.3)	(23.7)
Total		22/553	10/554	51/554	57/555	55/552	78/553
(%)		(4.0)	(1.8)	(9.2)	(10.3)	(10.0)	(14.1)

No answer and unknown answers are excluded

る医師主導治験を実施し，3歳以上13歳未満の年齢区分では既承認用量接種群における抗体産生が低かったことを報告している[15]．田村らはわが国の従来の規定により1歳未満の乳児と1歳児にワクチンを接種しHI抗体価変動を比較し，40倍以上の抗体価獲得の割合はいずれの型も乳児では有意に低値で，4倍以上の抗体価上昇の割合，平均抗体価ともにA香港型では有意差を認めなかったが，Aソ連型およびB型においては乳児では有意に低値であったことから，乳児と1歳児での抗体反応の差は年齢差ではなくワクチン接種量の差を反映したものとして，乳児に対する接種量増量の必要性を指摘している[2]．本研究では，WHO推奨用量のみにより3歳未満を0.25mL接種群，3歳以上を0.5mL接種群として比較分析しており，従来法との直接比較及び年齢の影響は確認できていない．しかしながら，少なくともビケン製とサノフィ製とでWHO推奨用量接種による免疫原性，安全性に差がないことから，わが国のインフルエンザHAワクチンの効果が低いとされる原因の一つに接種量が関係していることが示唆された．

以上のことから，わが国における小児に対するインフルエンザHAワクチンの効果を高めるために接種量を増量することは妥当であると考えられた．

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A Study on the HA Amount of HA Influenza Vaccination on Efficacy and Safety in Infants

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We examined the efficacy and safety of inactivated influenza vaccine when the amount of HA influenza vaccination in children was increased to the dose recommended by the WHO. The purpose of this study was to obtain basic evidence to review the vaccination dose in Japanese children. HA influenza vaccine produced by the Research Foundation for Microbial Diseases of Osaka University (Biken) licenced in Japan was administered through vaccination at the international dose, and split HA influenza vaccine produced by Sanofi Pasteur corp. (Sanofi) was used as control. Children from 6 months to less than 13 years of age were registered, and vaccinated with doses of 0.25mL or 0.5mL. Clinical symptoms during the influenza season were monitored to investigate vaccine efficacy, and information on adverse reactions was collected to evaluate safety profile. Paired serum HI and NT antibody titers were measured at pre first dose and post second dose of vaccination.

Both HI and NT antibody titers for H1N1 subtype were satisfactory elevated after administration of both vaccines. Elevation of the NT antibody titer for the H3N2 subtype was observed for both vaccines, but the H3N2 HI antibody titer for the Biken vaccine was not so high. For the subtype B virus, the NT titer had a better response than the HI titer for both vaccines. As only the H1N1 virus was prevalent in the area during the study period, we performed factor analysis concerning influenza contraction only for the H1N1 antibody titer. An HI titer of 1 : 40 or more at post-vaccination was a significant factor to lower the risk of influenza contraction. The relative risk for fever among children with an HI titer of 1 : 20 or less was significantly higher than those with an HI titer of 1 : 40 or more. Children with a higher HI titer had better prevention against fever, so that both vaccines were considered to be effective.

As for the appearance of adverse reactions, both vaccines were considered to be safe. From the above-mentioned results, vaccination with the Japanese Biken vaccine at an international dose was thought to be an effective and safe procedure.

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乳幼児へのインフルエンザワクチン接種量の増量について

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はじめに

感染症の発症予防に特異免疫が関与している[1]。特異免疫は抗体で代表される液性免疫と，特異的T細胞が関与する細胞性免疫からなっている。一般に，特異的細胞性免疫の測定は困難であるので，特異免疫の評価は測定が容易な抗体が用いられている。なお，特異免疫に関与する細胞群には，免疫記憶細胞と免疫実行細胞とがあり，抗体を産生する形質細胞や特異的細胞性細胞傷害に関与するCD8$^+$細胞は免疫実行細胞である。

免疫プライミングとは免疫がない人に免疫記憶細胞と免疫実行細胞を誘導することであり，免疫ブースティングとは免疫実行細胞の数を増加させ抗体を高めることである。不活化ワクチンではプライミングとブースティングの組み合わせが大切である。免疫実行細胞が成熟するのに6か月必要である。このため，プライミング終了6か月後以降に追加接種すると効果的なブースティングが認められる。また，一度ブースティングがかかると，4週間程度の短期間に追加接種を行ってもさらなる抗体上昇は認められない[2]。

本稿では，免疫学およびインフルエンザウイルス感染症（インフルエンザ）の臨床的特徴に基づき，小児接種量が増量された理論的背景について概説する。

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I．インフルエンザの特徴

1．インフルエンザは局所性ウイルス感染症

ウイルス感染症には，インフルエンザのように感染した局所でウイルスが増殖して症状が出現する局所性ウイルス感染症と，麻疹や水痘のように感染した局所で増殖したウイルスがウイルス血症により全身に拡がり，親和性臓器で増殖して症状が出現する全身性ウイルス感染症とがある。局所性感染症の発症予防には，局所粘膜で働く分泌型IgA抗体と血中から滲み出るIgG抗体が関与しているが，感染によるブースティングが働くまでに症状が出現するため，発症予防には高い抗体価が必要である。インフルエンザにおいて赤血球凝集抑制（hemagglutination inhibition：HI）抗体40倍は成人50%の，HI抗体160倍は成人90%の発症予防抗体価である[1]。小児でもHI抗体価が高いほど，優れた発症予防効果が認められている[3]。

2．インフルエンザウイルスの変異と抗体価

インフルエンザウイルスは変異しやすいウイルスであり，とくにA/H3N2はA/H1N1やB型と比べても変異が早いウイルスである。そのシーズンのワクチン株と3管（8倍）以上抗原性が変異した場合，変異ウイルスの出現と定義されている。抗原性が3管ずれた変異ウイルスが流行すると，ワクチン株に対するHI抗体価160倍は，流行株に対してはHI抗体価20倍に3管低下することになる。高い抗体価を保有していると，変異ウイルスに対しても発症予防に働くが，抗体価が低いと感染した場合，発症する危険性が増加する。

以上，インフルエンザの病態およびインフルエ