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Cell-cell fusion induced by measles virus amplifies the type I interferon response

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Measles virus (MeV) infection is characterized by the formation of multinuclear giant cells (MGC). We report that IFN-β production is amplified in vitro by the formation of virus-induced MGC derived from human epithelial cells or mature conventional dendritic cells (mDC). Both fusion and IFN-β response amplification was inhibited in a dose dependent way by a fusion inhibitory peptide after MeV infection of epithelial cells. This effect was observed at both low and high multiplicity of infection. While in the absence of virus replication, the cell-cell fusion mediated by MeV H/F glycoproteins did not activate any IFN-α/β production, an amplified IFN-β response was observed by infecting H/F-induced MGC with a non fusogenic recombinant chimerical virus. Time-lapse microscopy studies revealed that MeV-infected MGC from epithelial cells have a highly dynamic behavior and an unexpected long lifespan. Following the cell-cell fusion, both of the RIG-I and IFN-β gene deficiencies were trans-complemented to induce an IFN-β production. IFN-β and IFN-α production were also observed in MeV-infected immature and mature DC (iDC and mDC). In contrast to iDC, MeV infection of mDC induced MGC which produced enhanced amounts of IFN-α/β. The amplification of IFN-β production was associated with a sustained nuclear localization of IFN regulatory factor 3 (IRF-3) in MeV-induced MGC derived from both the epithelial cells and mDC, while the IRF-7 up-regulation was poorly sensitive to the fusion process. Therefore, MeV-induced cell-cell fusion amplifies IFN-α/β production in infected cells and this indicates that MGC contribute to the antiviral immune response.
INTRODUCTION

Measles virus (MeV) is an important human pathogen responsible for ~345 000 of deaths in 2005 (http://www.who.int/mediacentre/factsheets/fs286/en/). However, most humans clear this viral infection provided that they have a functional cellular and adaptive immunity (19). MeV infection begins in the respiratory tract and then spreads to local lymphoid tissues, where virus replication can occur in macrophages and possibly conventional dendritic cells (cDC) (14, 17, 22, 49, 70). This allows MeV spreading to other lymphoid organs and to the whole body. MeV induces a cytopathic effect characterized by the fusion of neighboring cells into multinucleated giant cells (MGC). In vivo, seven days after MeV infection of rhesus monkeys, MGC are found in respiratory and genitourinary tract, as well as in the esophagus and skin epithelia (47). In addition, a specific subset of infected MGC called Warthin-Finkeldey cells (WFC), initially described in infants dying of acute measles, is found in primary and secondary lymphoid organs (19). WFC are usually observed in germinal centers and interfollicular areas of secondary lymphoid organs and in the thymus. They are heterogeneous and display either B or T cells markers (54), although macrophage and DC markers have not yet been investigated. In vitro, MeV infection of human cells, including primary epithelial cells and cDC, induces the formation of MGC also referred to as syncytia (17, 78, 82). MeV-induced cell-cell fusion is governed by the interaction of the viral envelope H and F glycoproteins with the cellular receptors CD46 and CD150, which are expressed ubiquitously and solely on immune activated cells, respectively (19).

The hallmark of the immune response to a viral infection is the rapid production of a range of cytokines, most prominently type I IFN (IFN-α/β). The IFN-α/β enable cells to be protected against viral infection via pleiotropic activities such as inhibition of protein synthesis and cell
proliferation, and enhancement of infected cell apoptosis (reviewed in (5, 21)). They also activate Natural Killer (NK) cells and cytotoxic T cells (CTL) that are capable of eliminating the viral pathogen by killing infected cells. The IFN-α/β can act directly on CTL or indirectly by inducing the maturation of cDC, which facilitates cross-presentation of viral antigens to CTL (42, 43). The single IFN-β gene and most of the IFN-α genes differ in their promoter region, the former is activated by IFN regulatory factor 3 and 7 (IRF-3/IRF-7) heterodimers (56), whereas only IRF-7 homodimers (25) and/or IRF-7/IRF-8 heterodimers as recently reported in mouse DC (75), can activate the latter. The recognition of peculiar danger molecular motifs of viruses is mediated by host pattern recognition receptors (PRR) (36), which can recognize virus nucleic acids. Two groups of PRR are involved in IFN production in DC, the Toll-like receptors (TLR) and the RIG-like receptors (RLR). To date, TLR are mainly responsible for IFN-α production by plasmacytoid DC (pDC) via TLR7 and TLR9 (1), which respond to viral nucleic acids within the endosomal compartment. Nucleic acid recognition results in the activation of the IFN-α genes through the phosphorylation and the nuclear translocation of IRF-7 (26, 37). Indeed, in comparison with other cells, pDC express high level of IRF-7 (8, 30) and this allows pDC to produce 100- to 1000-fold more IFN-β and IFN-α than other cell types (26). TLR are also involved in IFN production by cDC via TLR3 after phagocytosis of infected cells (64). However, the IFN response in cDC principally relies on RLR (RIG-I and MDA-5) (34, 35), which are cytosolic PRR expressed in almost all nucleated cells and dedicated to respond to viral nucleic acids produced during replication ((28, 60, 87). MDA-5 and RIG-I recognized dsRNA (20) and 5’-triphosphate ended RNA (28, 60, 62), respectively. Accordingly, they recognize different types of RNA viruses, MDA-5 being activated by Picornaviridae and RIG-I by Flaviviridae, Orthomyxoviridae and Mononegavirales (20, 35). MeV activates RIG-I which recognizes the 5’-triphosphate end of the small RNA leader transcript (62). The activation of RLR induces the interaction with an adaptor.
protein called IPS-1 (also known as MAVS, VISA, CARDIF) that leads to NF-κB, IRF-3 and
IRF-7 phosphorylation and their transient translocation into the nucleus, resulting in the early
IFN-β gene induction (34, 57). In contrast to the short-living IRF-7, the more stable IRF-3 is
highly expressed in all cells (25). IFN-β is then secreted and binds to the IFNAR to produce late
IFN-β and all IFN-α subtypes. Indeed, early IFN-β induces the transcription of numerous genes
known as IFN-stimulated genes (ISG) (25), including IRF-7. The activation of this supplemental
source of IRF-7 allows the delayed production of a boost of both IFN-β and IFN-α, according to
a robust positive feedback loop, which amplifies the antiviral response (25). The crucial role of
the RLR in the IFN-α/β response and control of viral replication has been recently highlighted in
IPS-1 (or MAVS) deficient mice, which show normal IFN-α secretion by pDC (41, 74).
In the case of MeV, IFN-α/β production has been found after *in vitro* infection of various
human cell types including epithelial (from various tissues), endothelial, glial and PBMC (52, 84,
85). MeV propagates more efficiently in mature cDC (mDC) than in immature cDC (iDC) and
induces higher level of MGC formation in mDC (17, 66). Given the ability of MeV to induce
MGC formation in epithelium and secondary lymphoid organs, we aimed at investigating, both in
human epithelial cells and cDC, the role of MeV-induced cell-cell fusion on the regulation of
IFN-α/β production.
MATERIALS AND METHODS

Reagents. Antibodies used were: mAbs clone 55 (anti-H), clone 25, and 120 (anti-N), G28.5 (anti-CD40) and rabbit polyclonal anti-human IRF-3. The following reagents were used: Hoechst 33342 (Sigma), fusion-inhibiting peptide FIP Z-D-Phe-L-Phe-Gly-OH (Neosystem), rhGM-CSF and rhIL-4 (generously provided by Schering-Plough), rhIFN-β (Calbiochem, San Diego, CA), Draq5 (Alexis Biolabs).

Cells and phenotypic analysis. Human kidney epithelial 293T/17 cells (ATCC) expressing CD46 (293T/CD46+), HeLa cells, African green monkey Vero fibroblasts (ATCC), 293T/CD150+ (77), Huh7.5 cells, a subline of Huh7 defective in RIG-I (73), and human cortical TEC (P1.4D6 clone) from postnatal thymus (16) were maintained in DMEM medium supplemented with 10% FCS or as described in (16, 73). Monocyte-derived DC were generated in vitro from human blood (Etablissement de Transfusion Sanguine, Lyon, France) as previously described (17). After 6 days of culture in 200 ng/ml rhGM-CSF and 10 ng/ml rhIL-4, >95% of the cells were iDC. The mDC were derived by treating iDC for 48 h with 10 µg/ml of mAb anti-CD40. The cDC phenotyping was determined as previously described (17, 82).

MeV infection. MeV (Hallé strain) and recombinant chimerical MGV (72) were maintained in Vero cells. For MeV infection experiments, cells were seeded overnight at appropriate density in a 24-well plate according to the duration of the observation to avoid cell crowding. 293T and TEC were infected at MOI of 1, unless otherwise indicated, then treated or not with FIP. DC were infected at MOI of 0.1 with MeV as previously detailed (17, 83). As controls, all cells were
pulsed with a mock preparation corresponding to uninfected Vero cell supernatant. In some experiments, FIP (100 µg/ml) was added to the cDC cultures infected or not with MeV.

**Transient cell transfections.** 4x10^5 293T/CD46^+ cells were plated on 6-well plates (BD, Falcon). The day after, cells were transfected either with 1 µg pCXN2-F (expressing Edm-F) plus 1 µg pCXN2-EdH (expressing the CD46-binding H.Ed) or 1 µg pCXN2-KAH (expressing the H.KA from the wild type KA MeV isolate which does not bind to CD46) (79), or 2 µg of pBSK^+ carrier plasmid (Stratagene). All transfections were performed using Lipofectamine (Invitrogen) or Dreamfect (OZ Biosciences) according to manufacturers’ instructions.

**Time-Lapse.** 2x10^6 293T/CD46^+ cells were plated on a 6-well plate and infected with MeV at MOI of 1 as described above. After 24 h of culture in the presence of 10 µg/ml FIP, FIP was removed and the plate was placed on a 37°C heated in 5% CO₂ atmosphere (Carl Zeiss, Jena, Germany). Cells were imaged by Metamorph software v6 with a Coolscan HQ monochrome camera associated to timelapse microscope (Axiovert 100 M) and a x10 (numerical aperture 0.25) Plan-Apochromat objective (Zeiss). Meta Imaging Series 4.5 (Universal Imaging, West Chester, PA) was used to make Quick-Time movies from image stacks from metamorph software. One picture was made every 10 min for 60 hours and every second of movie represent 235.4 min (3.92 h) of culture (see Fig. S5video.mov). Images extracted from stacks were processed with Adobe Photoshop 6.0 (Adobe Systems, San Jose, CA).

**Co-culture experiments.** 8 x 10^4 Huh7.5 and Vero cells were plated in 24-well plates (Costar) and were infected, 24 h later, with MeV at MOI of 1. At 8 h.p.i., Vero cells were trypsinized and added to the Huh7.5 cell monolayers at a 1:1 ratio, in the presence or absence of FIP.
10 μg/ml of FIP. Cell-free supernatants were harvested at different times and frozen before analysis for IFN-α/β using a biological assay.

**IFN-α and IFN-β detection assay.** IFN-α/β contents in supernatants were determined using a bioassay as detailed elsewhere (84). IFN-α and IFN-β were determined by ELISA using the IFN-alpha kit from Bender MedSystems (detection limit = 8 pg/ml), and the IFN-beta kit from PBL Laboratories (detection limit = 250 pg/ml), respectively.

**RNA extraction, cDNA RT, and real-time QPCR analysis.** A detained procedure can be found for viral RNA quantification in (61). Primer sets for human IFN-β and IRF-7 mRNA quantitation were forward TGGGAGGATTCTGCATTACC, and reverse CAGCATCTGCTGGTTGAAGA primers, respectively. The primer sets were purchased from Search-LC (Heidelberg, Germany). Results were normalized according to the amounts of 18S rRNA, and expressed in mRNA copy number / 25 ng of total RNA.

**MTT colorimetric bioassay.** 4x10^3 293T/CD46^+ cells were plated in a 96-well plate (Costar). At 30 h after infection or transfection, cells were treated with 225 ng/well of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Sigma) to measure mitochondrial activity of metabolically active cells. 4 h latter, the supernatant was removed and the cells were lysed with 100 μl/well DMSO containing 0.04 N HCl. Absorbance was then measured at 490 and 650 nm (18).
Quantitative fusion assay. This assay is based on the conditional expression of β-185 galactosidase (β-Gal) under the control of the T7 polymerase promoter and was performed as 187 previously described (7).

Subcellular localization of GFP-IRF-3 proteins. 5x10^4 293T/CD46^+ cells, seeded in 24 189 well plate were infected or not with MeV at MOI of 1 for 2 h then transfected by the GFP-IRF-3 191 expression plasmid (46) using lipofectamine. 24 to 48 h latter, GFP-fluorescence in living cells 192 was analyzed with a Leica DM IRB microscope at a magnification of x400. The percent of IRF- 193 3-labeled nuclear was calculated by counting, within a microscope field, the total number of 194 nuclei (belonging to mononuclear cells or MGCs, labeled and unlabeled,) and the number of 195 nuclei labeled with GFP-IRF-3. In each condition, >100 nuclei were counted.

Stainings. For nuclear staining, cell monolayers were stained with Hoechst 33342 as detailed 197 previously (82) and the stained nuclei were observed using a Leica DM IRB microscope.

MeV H cell surface expression was detected using c155 mAb plus goat anti-mouse IgG 200 phycoerytrin-conjugated secondary antibody (GAM-PE), (Jackson ImmunoResearch 201 Laboratories) as previously described (82). MeV N intracellular detection was performed using 202 Cytofix/Cytoperm kit (Becton Dickinson, Parmingen) and 293T/CD46^+ cells were labeled using 203 biotinylated c125 mAb plus streptavidin-PE (Caltag Laboratories) as previously described (82). 204 After labelling, cells were analyzed using a FACScalibur flow cytometer (Becton Dickinson 205 Cellquest software). Integrated fluorescence was measured, and data were collected from at least 206 5,000 events.

Triple stainings were performed to visualized GFP-IRF-3 (green), MeV N protein (red) and 207 nuclei (blue) by confocal microscopy assays. 2.5 x 10^4 293T/CD46^+ cells were seeded onto pre-
coated poly-L-Lysine (Sigma, 10 µg/ml, overnight at 4°C) glass coverslips in a 24-well plate and incubated 20 min at 37°C. 12 h later, cells were infected with MeV at a MOI of 0.1, 1, 2 and 4, followed by transfection of the GFP-IRF-3 expression plasmid as described above, and then cultured in the absence or the presence of FIP at 10µg/ml. 48 h later, cells were fixed in 2% paraformaldehyde/PBS for 20 min at room temperature, treated with 0.1% Glycine/PBS for 10 min at room temperature, and permeabilized with 0.5% Triton X-100/PBS for 5 min at room temperature. After washes with PBS, the fixed cells were blocked in BSA, human and goat sera, Triton X-100/PBS overnight at +4°C. Cells were then incubated with c1120 anti-N mAb for 90 min at +4°C. Cells were washed 3 times for 5 min in PBS before incubation with a GAM-Alexa 568 for 30 min at +4°C. After 3 washes in PBS, cells were mounted on glass slides with mounting medium (Dako) containing Draq5 as nuclear marker. Labeled cells were imaged with a confocal microscope (Zeiss LSM510, 1 µm) using a zoomed (x2) 63X (NA 1.4) PlanFluor objective. To prevent cross-contamination between fluorochromes, each channel was imaged sequentially using a multi-track recording module before merging.

Double staining was performed to visualize endogenous IRF-3 (green) and nuclei (blue) by Axioplan2 Imaging microscopy assays. Briefly, 8x10^4 mDC were seeded onto pre-coated poly-L-Lysine glass coverslips in a 24-well plate and incubated 20 min at 37°C. 24 h later, cells were infected at a MOI of 0.1, and then cultured in the absence or the presence of FIP at 100µg/ml. At 3 d.p.i., cells were then fixed as for triple staining. Then, the fixed cells were blocked in BSA/serums/Triton X-100/PBS for 1 h at +4°C and incubated with rabbit anti-IRF-3 serum for 1 h at +4°C. Cells were washed 3 times for 5 min in PBS before incubation with a goat anti-rabbit IgG Alexa 488-conjugated secondary antibody (Molecular Probes) for 30 min at +4°C. After 3 washes in PBS, cells were incubated with 1 µg/ml of Hoechst 33343 for 15 min at room temperature before mounted on glass slides with mounting medium (Dako). Labelled cells were
analyzed with an Axioplan2 Imaging microscope (Zeiss, 0.3 µm) and then imaged by metamorph software V6. Images extracted from Z stacks were processed with Adobe Photoshop 6.0 software.

RESULTS

**MeV-induced cell-cell fusion amplifies IFN-β activation in epithelial cells.** While MeV induced a high number of syncytia containing numerous nuclei in cells expressing both MeV receptors (293T/CD46+CD150+), syncytia were smallest and less numerous in 293T/CD46+ cells expressing only CD46 (not shown). MeV infection of 293T/CD46+ cells led to the accumulation of F viral transcripts and to the induction of IFN-β gene expression (data not shown). The accumulation of viral F mRNA and the transcription of the IFN-β gene were both enhanced in 293T/CD46+CD150+ cells, compared to 293T/CD46+ cells (data not shown). Thus, the level of IFN-β activation was correlated to the virus transcription and/or to the cytopathic effects.

To decipher how each of these could influence the IFN response, we took advantage of the availability of a fusion inhibitory peptide (FIP). FIP efficiently blocks the MeV-induced syncytia formation without inhibiting the cell-to-cell virus spreading (19). Addition of FIP repressed both syncytium formation and IFN-α/β production in MeV-infected 293T/CD46+ culture, without affecting the proportion of cells expressing MeV-N protein (Fig. 1A, left histogram). Whereas the fusion was totally inhibited by FIP, the IFN-α/β production by day 3 (not shown) and 7 p.i. (Fig. 1A, left histogram) was inhibited by ~85-96%. This residual IFN-α/β production was significant, since no IFN-β could be detected in uninfected cells (data not shown). However, 293T cells expressing only MeV-Ed-H and -F glycoproteins readily fused into MGC, but did not secrete any
detectable IFN-α/β (data not shown). This indicates that cell-cell fusion, per se, does not activate the IFN-α/β response.

A recombinant chimerical virus, MGV, where H and F genes have been substituted by that of the VSV G glycoprotein (72), infected and propagated more slowly in 293T/CD46+ cells without inducing syncytium formation. Accordingly, it induced a low level of IFN-β production which was insensitive to FIP (Fig. 1A, right histogram), and equivalent to that observed in FIP-treated MeV-infected 293T/CD46+ cells. Thus, MGV only triggered a basal level of IFN-α/β response, in agreement with the observation of the low IFN-α/β inducing ability of a non-fusogenic MeV variant (48). To confirm the enhancing effect of cell-cell fusion on MeV-induced IFN-β response, we compared the effect of expressing in trans a fusing (HEdF) and a non fusing (HKAF) (79) glycoprotein combination on the IFN-β response induced by MGV infection. As expected, the expression of HKAF did affect neither virus nor IFN-β transcription (not shown). In contrast, when compared to HKAF, the expression of HEdF induced large syncytia into 293T/CD46+ cells, a minor increase of viral transcription and a significant increase in the IFN-β gene activation (Fig. 1B). Furthermore, the enhancing effect on the IFN-β response was much more pronounced in conditions ensuring that every single got infected, i.e. at MOI of 4, with a ~25 fold enhancement of IFN-β mRNA accumulation, compared to a limited 2.5 fold increase in virus transcript accumulation.

When 293T/CD46+ cells were infected with fusogenic MeV at MOI ranging from 0.01 up to 4 and analyzed at 30 h.p.i., the viral transcription of the F messenger exhibited a dose-response curve between MOI of 0.01 and 1 then reached a plateau (Fig. 1C, upper histograms). The identical level of viral transcription between MOI of 1 and 4 suggested that some viral interference occurred. In addition, at MOI higher than 1, a cytotoxicity, increasing with the MOI used, was observed, that likely resulted in part from cell fusion from without (i.e. fusion between...
adjacent cells bridged by viral particles) (6), a reminder of viral induced hemolysis of CD46 expressing Vervet monkey red blood cells (59). Furthermore, syncytia formation was much reduced when compared to lower MOI likely because of the strong down-regulation of CD46 upon contact with the large amount of the hemagglutinin brought about by the high viral inoculum (39, 51). The level of IFN-β transcription followed the same dose-response between MOI of 0.01 and 1, to reach a plateau at MOI of 1, 2 and 4 (Fig. 1C, middle histograms). This correlation between viral transcription and IFN-β gene activation agrees with their parallel kinetics observed at MOI of 1 (62). Surprisingly, the production of IFN-α/β in the supernatant measured at 3 d.p.i. was almost identical between MOI of 0.1 and 4 (Fig. 1C, lower histograms). At MOI of 0.01, a small amount of IFN-α/β was detected only later at 7 d.p.i. (data not shown). The addition of FIP, which inhibited the formation of syncytia at all MOI (Fig. 1C), had minimal effects on viral transcription, but strongly inhibited IFN-β gene transcription and protein secretion (Fig. 1C, black columns), except at MOI of 4. Interestingly, in the presence of FIP, the amount of IFN released in the supernatant and the MOI correlated for MOI between 0.1 and 2 (Fig. 1C and data not shown). At MOI of 0.01, the level of IFN released detected at 7 d.p.i. was also inhibited by FIP (not shown). The lack of FIP inhibitory effect at MOI of 4 was likely reflecting the side effects of the too high viral load mentioned above.

Furthermore, after infection at MOI of 1, FIP inhibition of the cell-cell fusion observed at 30 h.p.i. and 3 d.p.i., was dose-dependent as assessed by nucleus staining with Hoechst 33342 (Fig. 2A, and data not shown), and the quantification assay using β-Gal as a reporter gene for intercellular fusion (Fig. 2B). A similar inhibition curve was also observed for the IFN-β mRNA (Fig. 2C and not shown). Strikingly, the best mathematical equation describing these two dose-dependent responses had similar slopes (-11) and ordinates at the origin (+42 and +39).
In summary, MeV replication triggers a basal IFN-β response independently of H/F proteins and MeV-induced cell-cell fusion robustly amplifies this response in a dose-dependent manner at both low and high MOI.

**High morphologic plasticity of MeV-induced MGC.** Viral induced fusion is usually correlated with apoptosis (13). As we observed an increased synthesis of IFN-β in MGC, we further analyzed the morphological plasticity of these cells. Thirty hours p.i., syncytia were found to be metabolically active, and able to convert MTT into intracellular formazan crystals, a hallmark of mitochondrial activity in viable epithelial cells (data not shown).

Studies by time-lapse microscopy over 60 hours of MeV-infected 293T/CD46<sup>+</sup> cells, showed the syncytia to be dynamic, exhibiting a morphology which varied with time (Fig. 3A, B and Fig. S3Avideo.mov). In the 1<sup>st</sup> stage, the initial flat adherent syncytium increased in size and nucleus contents (Fig. 3A, Adherent). Dynamic pseudopodia emerged from the syncytium to contact surrounding cells or syncytia. In a 2<sup>nd</sup> stage, the adherent syncytium retracted into highly refringent smooth balls (3<sup>rd</sup> stage), where nuclei were no longer visible, except upon examination under confocal microscopy of z-stacks after Hoechst staining (see below). Balls were highly mobile and rolled around. When they encountered surrounding healthy adherent mononuclear cells, they spread out into a secondary flat adherent syncytium with visible nuclei (4<sup>th</sup> stage). Then, the secondary adherent syncytium retracted again (5<sup>th</sup> stage), into an irregular ball with protruding blisters giving them a cauliflower appearance (6<sup>th</sup> stage), which rolled around. The duration of each stage was highly variable (see mean values on Fig. 3B), and most of the initial syncytia passed through stages 1, 2 and 3, half of them passed through stages 4 and 5 to reach stage 6, and another half directly passed from stage 3 to stage 6 (Fig. 3B). Of 17 initial syncytia recorded during 3 different experiments, none appeared to die before 60 hours. Furthermore,
when individual smooth or blistered balls were transferred onto a fresh uninfected 293T/CD46+ monolayer, they re-adhered. This suggests that syncytia may have an indefinite lifespan provided that they find within their vicinity a healthy cell monolayer (Fig. 3B, and data not shown). In contrast, when transferred to a plastic dish covered or not by collagen, both smooth and blistered balls became senescent and finally died to become floating and optically clear bubble-like structures (data not shown). Thus, MeV-induced syncytia are not prone to quickly die; instead, they may remain a viable entity. As controls, cell-to-cell fusion was observed neither in uninfected nor in FIP-treated MeV-infected 293T/CD46+ cells, indicating that none of the observed syncytium was due to merging senescent 293T/CD46+ cells (Fig. 3C and data not shown).

Cell-cell fusion brings together both danger activation signal and IFN-β gene to trigger IFN-β induction. We then studied whether MeV-induced fusion can trigger trans-complementation using Huh7.5 cells and Vero cells which have disabled RIG-I (73) and lack the IFN-β gene (11, 12), respectively. IFN-α/β was secreted and accumulated over time from both of the MeV-Huh7.5/Vero and MeV-Vero/Huh7.5 cell co-culture combinations, as syncytia were observed (Fig. 4). Moreover, the addition of FIP completely blocked both of the cellular fusion and the IFN-α/β production to undetectable levels. As expected, MeV infection of isolated Huh7.5 or Vero cells induced syncytia but did not trigger any IFN-α/β response. Thus, the RIG-I defect in human Huh7.5 cells and IFN-β gene defect in the simian Vero cells could be trans-complemented in fused cells allowing the triggering of the human IFN-β gene.

IRF-7 expression level is not influenced by MeV-induced fusion in epithelial cells. We then investigated whether the enhancement of IFN-β gene expression mediated by MeV-induced
cell-cell fusion correlate with the up-regulation of IRF-7. To this end, the level of IRF-7 transcripts was analyzed in 293T/CD46+ cells and in the primary thymic epithelial cells (TEC), which are IFNAR-competent, in the presence or the absence of FIP (Fig. 5). In both of these cell types, MeV infection resulted in the activation of the IFN-β and IRF-7 genes. However, the level of IRF-7 transcripts remained unchanged when syncytia formation was blocked by FIP treatment, and did not correlate with the IFN-β mRNA level. Thus, the amplification of IFN-β activation by MeV-induced cell-cell fusion is not directly or solely controlled by the level of IRF-7 expression.

MeV-induced cell-cell fusion amplifies IFN-α and IFN-β responses in human mDC, but not iDC. The phenomenon of the amplification of the IFN-α/β response by MeV-induced cell-cell fusion was then examined in human monocyte-derived DC. More than 95% of iDC and mDC were CD1a+ and CD14− (data not shown). While the immature phenotype was confirmed by the low or negative expression of MHC class II, CD83, CD40, CD80 and CD86, mDC expressed high level of these molecules (data not shown). In agreement with previous reports (17, 50), both iDC and mDC, which have a CD46+CD150Low and CD46+CD150High phenotype, respectively (Fig. 6A) (50), were sensitive to MeV infection as shown by MeV-F transcription (Fig. 6B). However, the sensitivity to infection differed between iDC and mDC. MeV replication was faster in mDC in comparison with iDC with ~230-fold higher transcription at 3 d.p.i. (Fig. 6B). While the iDC only poorly fused, the MeV-infected mDC contained numerous giant MGC (Fig. 6), which expressed both viral proteins and mDC markers ((17), and data not shown). The addition of FIP to the cDC did not significantly affect the MeV-F transcription in the iDC and only partially reduced the MeV-F transcription in the mDC (Fig. 6B), while FIP efficiently inhibited the formation of MGC (Fig. 6). We then investigated the IFN-α/β production in cDC following MeV infection. MeV-infected iDC secreted significant levels of bioactive IFN-α/β (Fig. 6C) and
IFN-α (Fig. 6D). No IFN-β production was detected (Fig. 6E), even though IFN-β transcripts were observed (Fig. 6F), because of either the limited sensitivity of the ELISA, or the consumption of IFN-β by MeV-infected iDC (25). According to the very low level of cell-cell fusion observed within MeV infected iDC, the addition of FIP did not affect the IFN-α/β production (Fig. 6C and D). The MeV infected mDC produced significant levels of bioactive IFN-α/β, IFN-α, and IFN-β (Fig. 6C-E) and IFN-β mRNA (Fig. 6F). Both the MGC formation and IFN-α/β production by MeV-infected mDC was strongly inhibited in the presence of FIP (Fig. 6C-F). Thus, the MeV infection induces IFN-α/β responses in both of the iDC and mDC. However, the virus-induced cell-cell fusion amplifies both the IFN-α and IFN-β production only in mDC. Interestingly, MeV infection induced an IRF-7 up-regulation in the iDC, but not in mDC (Fig. 6G). Furthermore, FIP did not significantly affect the expression of IRF-7 in both DC types (Fig. 6G).

As a control, the non fusiogenic chimerical virus MGV was also used to infect iDC and mDC. MGV only induced, in both iDC and mDC, a basal IFN response, which was not sensitive to FIP (data not shown). We noticed that, contrary to MeV, MGV replicated better in iDC than in mDC (Fig. 6A) and, accordingly, induced a stronger IFN-β gene transcription in iDCs. We speculate that the stronger endocytosis ability of iDC over mDC can favor MGV entry, which relies on the acidic endosomal pathway mediated by the VSV-G glycoprotein.

Thus, MeV infection induces IFN-α/β responses in both iDC and mDC. However, the virus-induced cell-cell fusion amplifies both IFN-α and IFN-β production only in mDC.

Sustained nuclear translocation of IRF-3 in MeV-induced MGC derived from epithelial cells and mDC. MeV infection induces transactivation of the IFN-β gene through the phosphorylation and nuclear translocation of IRF-3 (65). Therefore, we analyzed the changes in
the subcellular IRF-3 localization using a GFP-tagged IRF-3 (GFP-IRF-3) transfected into 293T/CD46<sup>+</sup> cells. As expected (46), the GFP-IRF-3 was localized exclusively within the cytoplasm of uninfected cells (Fig. 7A, mock). After MeV infection, nuclei from a single syncytium exhibited a diverse level of GFP-IRF-3 staining, thus looking asynchronous, and a large proportion of syncytia contained nuclear IRF-3 whichever their stage. In addition, few mononuclear MeV-infected cells surrounding outside MGCs displayed nuclear localization of GFP-IRF-3 (not shown). In the absence of FIP, 50±24% of the nuclei belonging to MGC were labeled with GFP-IRF-3, whereas, only 6±7% of the small amount of single cells, which remained outside MGC, had their nuclei labelled (Fig. 7A, MeV). In the presence of FIP, although most cells were infected (see Fig. 1A, 7B and S7B), nuclear translocation of GFP-IRF-3 was observed only in 4±2% of them (Fig. 7A, MeV+FIP, 2α=0.01 when compared to 50±24% of nucleus labelling in the absence of FIP. In all cases, the nuclei were intact, including those in syncytia, as shown by Hoescht staining (Fig. 7A).

Confocal analysis of GFP-IRF3 distribution in 293T/CD46<sup>+</sup> cells infected with MeV at MOI of 1 and 2 confirmed the high rate of nuclear IRF-3 labeling in MGC compared to mononuclear cells, and the presence of cytoplasmic N protein in all but rare cells, (data not shown). At the lower MOI of 0.1, similar results were obtained. Upon confocal analysis, many nuclei within MGC were labeled with GFP-IRF-3, whereas GFP-IRF-3 remained excluded from the nuclei of non infected cells (Fig. 7B, and data not shown). When the fusion was prevented by the addition of FIP, only few mononuclear cell nuclei displayed GFP-IRF3 labelling, although most of them were clearly infected as shown by cytoplasmic N labeling (Fig. 7B red dots and patches, and data not shown). Again, GFP-IRF-3 was translocated into the nuclei of MGC at all stages of the adherent-ball cycle described in Fig. 3 (Fig. 7B and data not shown). Interestingly, the distribution of both N and GFP-IRF-3 tended to change with the morphological stage of the MGC. When the syncytium was flat and adherent, GFP-IRF-3 showed dotted and/or reticulated...
distribution in the nuclei and N protein had a dotted cytoplasmic distribution. When the syncytium underwent retraction into a smooth ball, the intensity of the nuclear GFP-IRF-3 labeling became stronger and more diffuse and N protein tend to aggregate further into larger patches at the periphery of the cytoplasm (Fig. 7B, and data not shown). Thus, the amplification of IFN-β production induced by MeV-mediated fusion correlates with a sustained and strong nuclear translocation of IRF-3 within MGC at all their morphological stages.

Localization of endogenous IRF-3 during infection of mDC was also studied. As expected, endogenous IRF-3 distributed exclusively within the cytoplasm of uninfected mDC (Fig. 7C, mock, left panels). At 3 d.p.i., IRF-3 (green) staining of many intact nuclei (blue stain) in syncytia MeV-infected mDC was observed (Fig. 7C, right panels, and supplemental material Fig. S7Cvideo.mov). Interestingly, the distribution of endogenous IRF-3 in the nuclei of mDC-derived syncytia looked very similar to that of GFP-IRF-3 in nuclei of 293T-derived syncytia (compare Fig. 7C with 7A, B and data not shown). Addition of FIP strongly inhibited both MeV-infected MGC from mDC and nuclear localization of endogenous IRF-3 (Fig. 7C, middle panel). Thus, endogenous IRF-3 tended to remain translocated into nuclei within MGC from MeV-infected mDC in agreement with the sustained nuclear translocation of exogenous GFP-IRF-3 within MGC derived from epithelial cells.

DISCUSSION

We report here that MeV-induced MGC or syncytia derived from epithelial cells and mDCs are metabolically active, long living and display both sustained nuclear translocation of IRF-3 and enhanced activation of the IFN-β gene. (i) There is a correlation between the fusogenic activity of MeV strain and IFN-β production. (ii) The fusion enhancing effect is observed at both
low and high MOI. (iii) The fusion inhibitory peptide FIP inhibits, in a dose dependent manner, both cell-cell fusion and IFN-β production. (iv) Cell-cell fusion mediated by MeV-H and -F glycoproteins does not activate any IFN-β response in the absence of viral infection, but does it after infection with MGV, a non-fusiogenic chimerical virus. (v) The cytosolic PRR RIG-I and the IFN-β gene are trans-complemented during the fusion process. (vi) Although both iDC and mDC are infected by MeV, only mDC undergo massive cell-cell fusion. In the mDC, a robust IFN-α/β production is mediated by MeV-induced cell-cell fusion (vii) In both epithelial cells and mDC, the fusion enhancing effect on IFN-β response appears to be mediated by a sustained nuclear IRF-3 localization, but does not directly correlate with the up-regulation of IRF-7 expression. (viii) In response to MeV, iDC also produces an IFN-α/β production, but it is independent of the fusion process and likely amplified via IRF-7. Altogether, our results indicate that the MeV-induced MGC in epithelial cells and mDC are important sources of IFN-α/β and that the fusion can mediate an enhancement of IFN-α/β production without modulating the expression of IRF-7.

Cell-cell fusion is a hallmark of many viral infections, and resulting MGC were thought to be short lived. Indeed, syncytia induced by the HIV-1 glycoprotein died by apoptosis by at least three different mechanisms: transient lipid exchange, activation of several kinases and transcription factors and contagious apoptosis (58). Surprisingly, MeV-induced MGC from epithelial cells were found to be viable and dynamic entities, capable of transducing intracellular signals throughout their morphological stage changes. Thus, from our in vitro observations, we can predict that the physiopathological MGC (WFC) observed in lymph nodes and thymus from MeV-infected children and primates should have a rather long lifespan in vivo (54, 76). However, as described by us and others (13, 58, 63, 82), syncytium apoptosis finally occurs, probably depending on cellular environment deprivation.
In non-pathological situations, the contents of a cell nucleus should be tightly regulated to ensure that every cell harbors a single nucleus. Notable exceptions are the fusion of cellular precursors undergoing a specific maturation process, such as the myotubes, the osteoclasts, and the syncytiotrophoblasts. In the two latter cases, a survival program is turned on (15, 31). Whether such a mechanism occurs for the survival of MeV-induced MGC remains to be determined. Strikingly, both of the MeV-induced MGC and syncytiotrophoblasts need to recruit fresh mononuclear cells in order to survive (29). Furthermore, given that IFN-β is used as a retro-control feedback to limit the size of the osteoclasts by preventing further recruitment of new mononuclear cells (9), it could also regulate the dynamics of MGC formation induced by MeV as observed for other viruses (53, 80, 81, 86).

The MeV induced cell-cell fusion results in MGC harboring an important function in the innate immunity, and it could be questioned if the fusion per se acts as an activation signal. Indeed, the artificial fusion of a human cell line with chicken erythrocytes results in the activation of both human and chicken IFN-β (23), the latter being indicative of a reactivation of the dormant chicken erythrocyte nucleus. The MeV induced cell-cell fusion is mediated by the H binding to the CD46 or CD150 cellular receptor which results in the activation of the fusion F protein (19). The H binding to CD46 has been reported to activate the IFN-β response and NO• production in murine macrophages expressing human CD46 (33). In human epithelial cells, the H/F and CD46-mediated fusion per se was unable to trigger the IFN-β response, which required virus transcription (62). Likewise, we can exclude that the interaction of H with TLR2 is involved in the IFN-β activation since (i) the signaling downstream to TLR2 occurred independently of the F glycoprotein, (ii) the use of a wild type MeV with H protein unable to bind to CD46 and TLR2 (4) gave similar results (not shown), and (iii) the TLR2 signaling pathway is not linked to IFN-
MeV infection of human epithelial cells triggers the production of IFN-β, which is differentially regulated in mononuclear cells and MGC. At the beginning of the viral infection, the activation of IFN-β occurs in MeV-infected mononuclear cells where the cytosolic RIG-I is activated upon recognition of the 5’-tri-phosphate end of MeV leader RNA (62), and this results in the activation of IRF-3. Then, IRF-3 undergoes phosphorylation, homo- or heterodimerization, nuclear translocation, fixation on IFN sensitive responsive elements (ISRE) and degradation by the ubiquitin-proteasome pathway (3, 65). In a later phase, the amplification of IFN-β production in MeV-infected mononuclear epithelial cells can be mediated by the classical IFNAR/IRF-7-dependent positive feedback, as described for other viruses (27), since IRF-7 is up-regulated after MeV infection. In contrast, the robust IFN-β production mediated by MGC from epithelial cells could not be explained solely by the IRF-7 up-regulation, because the latter was poorly sensitive to the fusion process. As described for an infection with respiratory syncytial virus, IRF-3 nuclear translocation occurs early within few hours after infection, then it drops rapidly within 15 hours because of the anti-interferon activity of non structural proteins (71). Therefore, the presence of a high IRF-3 nuclear translocation within MGC at a late time (30 h.p.i.) of the MeV infection is unexpected and supports an essential role of IRF-3 in the MGC-mediated amplification of the IFN-β production. It is possible that MeV proteins with IFN antagonist activity are diluted out upon fusion of MGC with uninfected cells, thus allowing a stronger and more sustained IFN production. It remains to be determined if the sustained IRF-3 nuclear localization within MGC occurs as phosphorylated IRF-3 homodimers or IRF-3/IRF-7 heterodimers.
What could be the mechanism, which enables cell fusion to boost the IFN-β activation?

At low MOI (virus to cell ratio <1) of infection, we propose the following model. Since the RIG-I and IFN-β gene locus are trans-complemented during the cell-cell fusion, syncytia formation can boost IFN-β transcription by bringing uninfected cells into contact with viral PAMPs. At a given time, the level of IFN-β activation results from the balance between available trigger viral RNA (PAMPs), RIG-I (PRR), pathway components (i.e. IRF-3) and viral IFN antagonists. The sustained nuclear localization of IRF-3 in the nuclei within syncytia at low MOI is compatible with the continuous recruitment of non infected cells which can result either in a weaken concentration of viral antagonists, and/or the recruitment of “naive” RIG-I/pathway components molecules by MeV leader RNA which would be produced in excess over the amount of RIG-I/pathway components available in a single cell. At high MOI, an alternative model should be made since all individual cells get infected prior to the fusion event and the fusion-mediated amplification of the IFN-β response is even higher (see Fig. 1B). The IRF-3 nuclear translocation could be sustained within MeV-induced MGC, because of a synergistic activity such as stabilization of phosphorylated IRF-3 by activation of the DNA-dependent protein kinase (DNA-PK) (32). This will require further investigations. Presently, our data thus argue for two non exclusive mechanisms involved in the fusion enhancing effect on IFN-β activation, one, evidenced at low MOI, is the recruitment of non infected cells to MeV-infected MGC, and the other, at high MOI, is a synergistic effect of cell fusion and virus infection. In both cases, there is a sustained nuclear translocation of IRF-3 within MGC, the underlying mechanism of which remains to be more deeply examined. In every case, the amplification of IFN-β response by MGC derived from MeV-infected epithelial cells upgrades the alert level of the innate immune response against viral infection in peripheral tissues.
During natural infection, MeV infects lung epithelial cells and/or resident iDC in epithelia and mucosa, and likely induces local IFN-α/β production, which could limit MeV replication (45). Then, infected iDC can migrate and disseminate the virus to the draining lymph nodes. There, they can be stimulated via CD40L by encountering naive T lymphocytes and become activated and more permissive to MeV replication as shown experimentally (66). Because the mDC are prone to fuse with surrounding cells, they form MGC, which could correspond to the WFC found in lymphoid organs. This results in a high virus progeny, which can propagate throughout the body. There are several examples of IFN-α/β production by human or mouse iDC (2, 34, 35) infected in vitro by few viruses, including MeV (38). Here, we demonstrate that upon MeV infection, both of the iDC and mDC produce IFN-β and IFN-α in vitro. The iDC display low permissiveness to MeV infection, and rapidly produce high levels of IFN-α and IFN-β independently of cell-cell fusion, probably through IFNAR/IRF-7 signaling as judged by the up-regulation of IRF-7 expression. Since, IFN-α/β is quickly produced and secreted by iDC after infection with MeV (not shown), IFN-α/β can protect cells against the propagation of MeV and strongly limit the formation of MGC. As iDC are present in peripheral tissues and secrete IFN-α/β, they can contribute to the establishment of the innate antiviral state by enhancing cytotoxicity of NK cells and activating macrophages (10, 40). In addition, the iDC constitute a critical link between innate and adaptive immunity (44, 67). Indeed, the IFN-α/β induce the up-regulation of co-stimulatory molecules CD80, CD86 and CD40 on cDC (24), and the expression of TRAIL on iDC, which become cytotoxic (83). Thus, the IFN-α/β produced by MeV-infected iDC is a signal which upgrades the alarm level of the cellular innate immunity for detecting the invasion of a possible pathogen.

In contrast to the iDC, the mDC are highly susceptible to MeV infection, form large MGC and produce high levels of IFN-α/β. The IFN-α/β is therefore less efficient in limiting MeV
growth and MGC formation within the mDC than within the iDC. The opposite phenotypes of iDC and mDC could originate from different relative kinetics of the infection and the innate antiviral response. Indeed, when the strength of the initial activation of IFN-β is too low compared to the virus growth kinetics, the rapid accumulation of MeV encoded anti-IFN V and possibly C proteins can block the intracellular IFNAR signaling pathway (55, 69), and this paves the way for unlimited virus growth. We therefore favor that the MGC can be promoted or repressed according to the respective speed and strength of virus growth and IFN-α/β production.

Upon infection, the mDC produce IFN-α/β mostly from MGC, without any IRF-7 up-regulation. This suggests that the IFNAR/IRF-7 feedback loop is not directly involved. This data is in agreement with the down-regulation of IFNAR in cDC upon their maturation (68). As for epithelial cells, the robust production of IFN-α/β by the MeV-mediated MGC from mDC also correlated with a sustained activation of IRF-3. However, the induction of IFN-α independently of IRF-7 up-regulation in the MGC is questionable and the mechanism remains to be determined.

The IFN-α/β produced by MGC from MeV-infected mDC could rather be involved in the establishment of MeV-specific adaptive immune response in the secondary lymph nodes. By providing high viral antigen load and IFN-α/β-dependent enhancement of the cross-priming to T cells (43), the paradoxical accumulation of virus and IFN-α/β within the MeV-mediated MGC probably contributes to the stimulation of the MeV-specific adaptive immune response, which will finally clear the virus from the organism. Finally, because of the different abilities of various laboratory, vaccine and wild type MeV to counteract the cellular innate immunity, the virus strain dependency of cell fusion-mediated amplification of the IFN-α/β response is also currently under investigation.

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REFERENCES


5. Bonjardim, C. A. 2005. Interferons (IFNs) are key cytokines in both innate and adaptive antiviral immune responses--and viruses counteract IFN action. Microbes Infect 7:569-78.


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42. Lapenta, C., S. M. Santini, M. Spada, S. Donati, F. Urbani, D. Accapezzato, D.
Franceschini, M. Andreotti, V. Barnaba, and F. Belardelli. 2006. IFN-alpha-
conditioned dendritic cells are highly efficient in inducing cross-priming CD8(+) T cells

D. F. Tough. 2003. Cross-priming of CD8+ T cells stimulated by virus-induced type I


measles virus replication in human peripheral blood mononuclear cells. Apmis 100:125-
31.

phosphorylation of the IRF-3 transcription factor regulates nuclear translocation,
transactivation potential, and proteasome-mediated degradation. Mol Cell Biol 18:2986-
96.

and the immunized host. Virology 233:74-84.


2000. Lymphatic dissemination and comparative pathology of recombinant measles


Figure 1. Role of cell-cell fusion and MeV infection in the IFN-β response. (A) Syncytia formation scored as in Fig. S1, % of cells expressing MeV-N protein determined by flow cytometry, and IFN-α/β production at 7 d.p.i. after infection of 293T/CD46+ cells with MeV (left histogram), or recombinant chimerical MGV (right histogram), in the absence (dotted columns) or presence of 10 µg/ml of FIP (black columns). (B) Syncytia formation and accumulation of MeV-N (upper panel) and IFN-β (lower panel) transcripts at 23 h.p.i. in 293T/CD46+ cells transiently expressing HEdF (grid columns) or HKAF (checked columns), respectively. 293T/CD46+ cells were infected with MGV with different MOI, then they were transfected at 2 h.p.i. (C) Dose response relationship of MeV-F transcription and IFN-β mRNA accumulation at 30 h.p.i. and IFN-α/β secretion at 3 d.p.i. with the MOI of MeV used to infect 293T/CD46+ cells in the absence (dotted columns) or the presence of 10 µg/ml FIP (black columns). Data are mean values ± s.d. from two to three independent experiments. ND: not detected. † indicates cell cytotoxicity.

Figure 2. Similar dose-dependent inhibition of cell-cell fusion and IFN-β gene transcription by FIP. 293T/CD46+ (A and C) or HeLa (B) cells were infected with MeV MOI of 1 prior to the addition of increasing amounts of FIP. (A) Micrographs of adherent cells stained with Hoechst 33342 (magnification x400) at 72 h.p.i. Cells containing more than three nuclei were considered as syncytia (white arrows). (B) Dose-dependent inhibition of cell-cell fusion by FIP quantified by colorimetric β-Gal reporter gene expression assay. (C) Dose-dependent inhibition of IFN-β mRNA accumulation by FIP at 30 h.p.i. Data are means ± s.d. of triplicates.
Figure 3. MeV-induced syncytia are dynamic entities with extended lifespan. MeV-infected 293T/CD46+ were cultured overnight in the presence of FIP, then cultured in the absence (A, B) or the presence (C) of FIP (10 µg/ml) with recorded imaging for the next 60 h by time-lapse microscopy. Images at a magnification x10 were extracted from Fig. S3A video.mov and another video not shown at 12.83 h, 15 h, 16.66 h, 25.33 h, 32.5 h and 35.33 h recorded times. (B) The duration of each stage was evaluated and expressed as mean ± s.d. of 17 microscopic areas from three to four separate experiments. The frequency was estimated and indicated as the proportion (%) that underwent transition through a given stage.

Figure 4. Reciprocal trans-complementation of RIG-I and IFN-β deficient cells by MeV-induced fusion. RIG-I-deficient Huh7.5 or IFN-β-deficient Vero cells were infected with MeV at MOI of 1, and co-cultured 8 h later with uninfected Vero and Huh7.5 cells (ratio 1:1), respectively. The co-cultures were treated or not with 10 µg/ml of FIP. Cell-free supernatants were collected at 30 and 60 h.p.i. to measure IFN-α/β production. At the end of the co-culture, the cell monolayers were stained for fluorescent nuclei (magnification of x400) for counting within every syncytium indicated by arrows. Data are from one representative experiment out of two. ND: not detected.

Figure 5. Unlike IFN-β gene, IRF-7 gene expression does not correlate with cell-cell fusion. 293T/CD46+ cells (left panels) and TEC (right panels) were either treated with 1000 IU/ml of rhIFN-β or infected with MeV MOI of 1 and cultured in the absence or presence of FIP (10 µg/ml). Expression of IFN-β (upper dotted histograms) and IRF-7 (lower black histograms)
mRNA was quantified at 30 h.p.i. Data are from one representative experiment out of two or three. ND: not detected.

Figure 6. Mature, but not immature, DC exhibit fusion-dependent amplification of IFN-α and IFN-β responses. iDC and mDC were mock infected or infected with MeV strain at MOI of 0.1, in the absence (dotted columns) or the presence (black columns) of FIP (100 µg/ml). Syncytia formation was scored for each condition as described in Fig. S1. (A) CD150 expression on iDC and mDC cultures was analyzed by flow cytometry. (B) MeV-F transcript accumulation in iDC and mDC cultures was measured at 3 d.p.i. (C) Secreted bioactive IFN-α/β in cell-free supernatants collected at 3 d.p.i. IFN-α (D) and IFN-β (E) were measured by ELISA at 3 d.p.i. Accumulation of (F) IFN-β and (G) IRF-7 transcripts at 3 d.p.i. in cDC cultures in the absence or the presence of FIP (100 µg/ml). Data are mean values from two to five separate experiments. ND: not detected.

Figure 7. Nuclear translocation of IRF-3 can be triggered within MeV-induced syncytia. (A) Nuclear translocation of GFP-IRF-3 within syncytia of 293T/CD46+ cells infected by MeV at MOI of 1. Microphotographs (magnification x400) showing morphology (upper panels), Hoescht labeled nuclei (middle panels) and GFP-IRF-3 labeled nuclei (bottom panels) at 30 h.p.i. Micrographs (magnification of x400) of uninfected cells (mock) and cells infected with MeV in the absence (MeV) or the presence of FIP (10 µg/ml, MeV+FIP) are shown. Data are from one representative experiment out of four. (B) Three-color overlays of confocal images showing the distribution of GFP-IRF-3 (green), N (red), nuclei (Draq5, blue) in 293T/CD46+ cells infected or not with MOI of 0.1 MeV in the presence or absence of FIP and transfected with GFP-IRF-3. Syncytium images were taken at three morphological stages, flat adherent, retracting and smooth
ball, respectively. The whole set of one-color images used to build the overlays is shown in the supplementary Fig.7SB. (C) Nuclear localization of endogenous IRF-3 in MGC derived from MeV-infected mDC. mDC were mock-treated (left panel, magnification x63) or infected with MeV at a MOI of 0.1 in the absence (middle panel, magnification x40) or the presence (right panel, magnification x63) of FIP (100 µg/ml). 3 d.p.i., mDC culture were stained with anti-IRF-3 (green, left panel) and with nucleus staining (Hoechst 33342, blue). Cells were analyzed with an axioplan microscope.

LEGENDS TO SUPPLEMENTAL DATA

Figure S3Avideo.mov. Time-lapse microscopy of MeV-infected 293T/CD46⁺ in the absence of FIP. Animation Format: 380x331 million; sequence, FPS 3,75; Reading: IPS 9; Flow: 9,46 mbits/sec; Duration: 9.26 sec; Size and actual size: 100%: 338x331 pixels (see material and methods and legends to Figure 3 for details).

Figure S7Cvideo.mov. Nuclear endogenous IRF3 localisation within MeV-induced MGC from mDC. z stak compilation of images from MeV-infected mDC in the absence of FIP (see material and methods).
Figure 1
Figure 2
**Figure 3**

(A) Stages of cell movement:
- **Adherent**: Stage 1
- **Retracting**: Stage 2
- **Smooth ball (Rolling)**: Stage 3
- **Adherent**: Stage 4
- **Retracting**: Stage 5
- **Blistered ball (Rolling)**: Stage 6

(B) Percentage and duration of each stage:
- Adherent: 100% > 14 ± 8 h
- Retracting: 81% 5 ± 5 h
- Rolling: 94% 6 ± 6 h
- Adherent: 44 - 56% Adherent 6.5 ± 7 h
- Retracting: 44 - 56% Retracting 4.3 ± 7.4 h
- Rolling: 100% Rolling > 19 ± 8.3 h

(C) Images illustrating the progression of cell movement through stages.
Figure 4
Figure 5
Figure 6
Figure 7 A,B
Figure 7C