Measles Virus Vaccine–Infected Tumor Cells Induce Tumor Antigen Cross-Presentation by Human Plasmacytoid Dendritic Cells

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Abstract

Purpose: Plasmacytoid dendritic cells (pDC) are antigen-presenting cells specialized in antiviral response. The measles virus vaccine is proposed as an antitumor agent to target and specifically kill tumor cells without infecting healthy cells.

Experimental Design: Here, we investigated, in vitro, the effects of measles virus vaccine–infected tumor cells on the phenotype and functions of human pDC. We studied maturation and tumor antigen cross-presentation by pDC, exposed either to the virus alone, or to measles virus vaccine–infected or UV-irradiated tumor cells.

Results: We found that only measles virus vaccine–infected cells induced pDC maturation with a strong production of IFN-α, whereas UV-irradiated tumor cells were unable to activate pDC. This IFN-α production was triggered by the interaction of measles virus vaccine single-stranded RNA (ssRNA) with TLR7. We observed that measles virus vaccine–infected tumor cells were phagocytosed by pDC. Interestingly, we showed cross-presentation of the tumor antigen NYESO-1 to a specific CD8+ T-cell clone when pDC were cocultured with measles virus vaccine–infected tumor cells, whereas pDC were unable to cross-present NYESO-1 after coculture with UV-irradiated tumor cells.

Conclusions: Altogether, our results suggest that the use of measles virus vaccine in antitumor virotherapy induces immunogenic tumor cell death, allowing pDC to mature, produce high amounts of IFN-α, and cross-present tumor antigen, thus representing a mode of recruiting these antigen-presenting cells in the immune response. Clin Cancer Res; 19(5); 1147–58. ©2012 AACR.

Introduction

Measles virus vaccine–based antitumor virotherapy is a new therapeutic approach to treat cancers (1, 2). It is based on the ability of a vaccine-attenuated live strain of measles virus, such as Edmonston or Schwarz strains, to preferentially infect and kill tumor cells. Measles virus vaccine uses mainly CD46 molecules to infect cells, whereas wild-type measles virus preferentially uses SLAM (CD150; refs. 3, 4). During cancer development, tumor cells are often selected to express high levels of CD46 molecules, which inhibit the complement system (5, 6). This CD46 overexpression makes the tumor cells less sensitive to lysis by the complement but renders them sensitive to measles virus vaccine infection. We and others have reported that several types of cancer are sensitive to this approach in vitro, such as mesothelioma (7), ovarian cancer (8), multiple myeloma (9), breast cancer (10), hepatocellular carcinoma (11), and melanoma (12). In vivo efficacy of this approach has also been reported in models of human tumor xenografts in immunodeficient mice (8–11). Phase I clinical trials against different malignancies are now in progress. In a first phase I clinical trial, intratumoral injection of low doses of measles virus vaccine to 5 patients with cutaneous T-cell lymphoma allowed stabilization of the disease in 2 patients and the observation of a partial response in one other (13). Results from a further phase I trial were published recently, in which patients with chemotherapy-resistant ovarian cancer were treated with intraperitoneal measles virus vaccine (14). Twenty-one patients were treated and improvements in serum tumor markers were noted in 5.
This promising efficacy of measles virus vaccine antitumor virotherapy is mainly a result of the lysis of tumor cells following measles virus vaccine infection, as shown by in vitro studies in immunodeficient mice (15, 16). In these immunodeficient mice, it has also been shown that measles virus vaccine infection triggers neutrophil infiltration of tumors, which probably participates in the efficiency of the antitumor virotherapy (17). Indeed, measles virus vaccine infection of tumor cells may also be able to trigger the immune system to induce an antitumor immune response.

We previously showed, in vitro, that mesothelioma cells killed by measles virus vaccine were able to produce danger signals, trigger the maturation of myeloid dendritic cells, and cross-present tumor antigens to CD8$^+$ T lymphocytes, whereas the same tumor cells killed by UV irradiation did not (7). Similar results were recently reported following lysis of melanoma tumor cells by measles virus vaccine (12).

Plasmacytoid dendritic cells (pDC) are a subset of dendritic cells involved in the antiviral immune response due to their expression of Toll-like receptors (TLR) specialized in the recognition of viral nucleic acids (TLR7 and TLR9; ref. 18). They respond to a wide range of viruses (inter alia influenza A virus, herpes simplex virus, HIV) in terms of activation and maturation by producing large amounts of type 1 IFN (IFN-$\alpha$, -$\beta$, -$\gamma$). They are also able to present viral antigens to CD8$^+$ and CD4$^+$ T cells when they are infected by a virus (19) and to cross-present viral antigens from virus-infected cells to CD8$^+$ T lymphocytes (20–23). It has also been shown that these pDC could play a beneficial role in the immune response against tumors (24, 25). As an example, in a mouse melanoma model, pDC activation and antitumor immune response were observed inside tumors by topical treatment with the TLR7 ligand, imiquimod (24).

Translational Relevance

Measles virus vaccine–based antitumor virotherapy is a new therapeutic approach to treat cancers. It is based on the spontaneous capacity of measles virus vaccine to infect and kill preferentially tumor cells. Phase I clinical trials against different malignancies are in progress with encouraging results. This promising efficacy of measles virus vaccine antitumor virotherapy is mainly due to the lysis of tumor cells following measles virus vaccine infection. However, it is likely that a part of this efficacy is due to the effect of measles virus vaccine–infected tumor cells on the antitumor immune response. In this study, we show that measles virus vaccine–infected tumor cells activate human plasmacytoid dendritic cells (pDC), a particular subset of dendritic cells specialized in the antiviral immune response. Human pDC are then able to cross-present a tumor antigen from measles virus vaccine–infected tumor cells to specific cytotoxic T cells. Our work thus suggests that measles virus vaccine–based antitumor virotherapy triggers an antitumor immune response, notably by the recruitment of pDC.

As measles virus vaccine is single-stranded RNA (ssRNA), pDC should be able to detect the measles virus vaccine infection of tumor cells, because of their intravacuolar TLR7 expression, which recognizes ssRNA. In addition, although it has been well described that human pDC are able to cross-present viral antigens, there is as yet no evidence that these antigen-presenting cells are able to cross-present tumor antigens.

In our study, we addressed, in vitro, the effect of tumor cell infection by measles virus vaccine Schwarz on the activation status of human pDC and their ability to cross-present a tumor antigen to a specific CD8$^+$ T-cell clone. We showed that, despite CD46 expression, pDC are not sensitive to measles virus vaccine infection. However, they are able to respond in vitro to measles virus vaccine by producing IFN-$\alpha$, with a greater sensitivity when interleukin (IL)-3 is added to the culture. We also showed that measles virus vaccine–infected tumor cells triggered pDC activation, notably IFN-$\alpha$ production, whereas UV-irradiated tumor cells did not. pDC activation was probably caused by the ssRNA of measles virus vaccine, which triggers TLR7 in the pDC endocytic compartment following phagocytosis of measles virus vaccine–infected tumor cells. Interestingly, we showed for the first time that human pDC cocultured with measles virus vaccine–infected tumor cells were able to cross-present the NYESO-1 tumor antigen to a specific CD8$^+$ T-cell clone. Our results suggest that, in addition to a direct tumor lysis effect, measles virus vaccine–based antitumor virotherapy may trigger an antitumor immune response by activating pDC.

Materials and Methods

Cell culture

The mesothelioma Meso13 cell line was established and characterized in our laboratory (26), the melanoma M18 was a kind gift from Dr. Labarriere [Institut National de la Santé et de la Recherche Medicale (INSERM) U892, Nantes, France], and the pulmonary adenocarcinoma A549 cell line was purchased from ATCC (Manassas, VA). All cell lines were cultured at 37°C, 5% CO$_2$ atmosphere in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin and 2 mmol/L L-glutamine (all reagents were purchased from Gibco-Invitrogen, Cergy-Pontoise, France). Cells were routinely checked for mycoplasma contamination, by PCR. A549 is HLA-A*2501+; A*3001+ (27). M18 is HLA-A*2902 homozygous as determined by PCR (data not shown).

Measles virus vaccine infection and UV irradiation

Live-attenuated Schwarz-strain measles virus vaccine and recombinant measles virus vaccine–enhanced GFP (MV-eGFP) were produced as previously described (7). Measles virus vaccine infection of tumor cells was conducted for 2 hours at 37°C with a multiplicity of infection (MOI) of 1 unless otherwise indicated. Viral inoculum was then replaced by fresh cell medium for 72 hours. For pDC infection and maturation experiments, measles virus...
UV-irradiated tumor cells were stained with PKH-67 and microscopy (Nikon). Measles virus vaccine–infected and ciences). pDC phagocytosis was observed by confocal analyzed by flow cytometry (FACScantoII, BD Biosciences) and conjugated, anti-HLA-DR-antibody (BD Biosciences) and cell-conjugate. pDC were stained by an HorizonV450–

Cocultures were washed with PBS–EDTA to dissociate the rhIL-3 (Sigma) or activated

Dendritic cell isolation and culture

pDC were obtained from healthy donor peripheral blood mononuclear cells (PBMC; Etablissement Français du Sang) as previously described (28). Briefly, pDC were first enriched by counterflow centrifugation and then purified by magnetic bead negative selection as recommended in the manufacturer’s protocol (Stemcell Technologies). The purity of untouched pDC was always greater than 96%. pDC (3 × 10^5 per mL) were maintained in culture with 20 ng/mL rhIL-3 (Sigma) or activated in vitro with a TLR-7 agonist, R848 (InvivoGen; 5 µg/mL). pDC were also cocultured with measles virus vaccine–infected alone, measles virus vaccine, and IL-3 (MOI = 1), or measles virus vaccine–infected or UV-irradiated tumor cells (pDC:tumor cell ratio, 1:1) without rhIL-3 or maturation agent. After 18 hours, culture supernatants and pDC were harvested for use. For the TLR-7 inhibition assay, we used immunoregulatory DNA sequences (IRS), which specifically inhibit signaling via TLR-7 (IRS661), at concentrations ranging from 0.1 to 1 µmol/L (Eurofins). As a control, we used CpG-A at 5 µg/mL to induce a TLR-9–dependent IFN-α secretion by pDC (InvivoGen).

Immunofluorescence and flow cytometry

The phenotypes of pDC were determined by immunofluorescence followed by flow cytometry. pDC were stained with monoclonal antibodies specific for CD40, CD86, HLA-DR (BD Biosciences), CD83 (BioLegend), and BDCA-4 (Miltenyi Biotec). pDC were gated as BDCA-4+/HLA-DR^+ cells, to differentiate them from tumor cells. Tumor cell death was measured by TO-PRO3 (Invitrogen) staining as recommended by the manufacturer. TO-PRO3 is a carbocyanine monomer nucleic acid with far-red fluorescence that enters only in dead cells and stains the DNA. Fluorescence was analyzed on FACScantoll (Becton Dickinson) using FlowJo software.

Phagocytosis assay

Measles virus vaccine–infected and UV-irradiated tumor cells were stained with PKH-67 according to the manufacturer’s protocol (Sigma) and cocultured with pDC, for 18 hours at 4°C or 37°C (1 dendritic cell:1 tumor cell). Cocultures were washed with PBS–EDTA to dissociate the cell-conjugate. pDC were stained by an HorizonV450–conjugated, anti-HLA-DR-antibody (BD Biosciences) and analyzed by flow cytometry (FACScantoll, BD Biosciences). pDC phagocytosis was observed by confocal microscopy (Nikon). Measles virus vaccine–infected and UV-irradiated tumor cells were stained with PKH-67 and then cocultured with pDC in 24-well plates containing poly-lysine glass slides, for 18 hours (pDC:tumor cell ratio 1:1). pDC were stained with uncoupled anti-HLA-DR (BD Biosciences). HLA-DR staining was revealed with a secondary anti-mouse IgG antibody coupled to Alexa Fluor 568.

Cytokine detection

IFN-α (MabTech) production was measured by ELISA on pDC culture supernatants according to the manufacturer’s instructions.

Cross-presentation assay

NYESO-1pos/HLA-A’0201neg melanoma (M18) and NYESO-1neg/HLA-A’0201pos pulmonary adenocarcinoma (A549) cell lines were measles virus vaccine–infected or UV-B–irradiated and cultured for 72 hours. They were then cocultured with HLA-A’0201pos pDC (pDC:tumor cell ratio, 1:1). After 18 hours, pDC were cocultured with the HLA-A’0201/NYESO-1(156-165)-specific CD8^+ T-cell clone, M117.167, for 6 hours in the presence of Brefeldin-A (Sigma). The M117.167 clone was obtained by cloning in a limiting dilution of tumor-infiltrating lymphocytes from a patient with melanoma. The clone was cultured as described (29). As control, we used pDC pulsed for 1 hour with 0.1 or 1 µmol/L NYESO-1(156-165) peptide and washed. Cells were then fixed with PBS containing 4% paraformaldehyde, for 10 minutes at room temperature, and permeabilized and stained with IFN-γ and CD8-specific antibodies (BD Biosciences), as previously described (30). IFN-γ production was analyzed by flow cytometry with a gate on CD8^+ T cells.

Real-time RT-PCR

One microgram of total RNA was reverse-transcribed using Moloney murine leukemia virus reverse transcriptase (InvitroGen). PCR reactions were conducted using Quantitect primers (Qiagen) and RT^2 Real-Time SYBR-Green/ROX PCR mastermix (Tebu-bio) according to the manufacturers’ instructions.

Statistics

GraphPad Prism (GraphPad Software Inc.) software using a nonparametric Mann–Whitney comparison test was used. P values less than 0.05 were considered to be statistically significant.

Results

Sensitivity of tumor cells and pDC to measles virus vaccine infection

During infection, measles virus vaccine enters cells mainly via the CD46 and, to a lesser extent, CD150/SLAM (3, 4). In a first experiment, we studied the expression of these 2 major measles virus vaccine receptors, CD46 and CD150/SLAM, on pDC, melanoma (M18), mesothelioma (Meso13), and pulmonary adenocarcinoma (A549) cell lines (Fig. 1A). We observed CD46 expression on all cell types, with higher expression on Meso13 and A549. About CD150/SLAM expression, we found a positive expression on the melanoma cell line, M18. These results suggest that
all these cell types may be sensitive to measles virus vaccine infection, as they all express CD46.

We then studied the sensitivity to measles virus vaccine infection of these 4 cell types using a recombinant measles virus vaccine encoding the GFP (MV-GFP). Seventy-two hours after exposure to measles virus vaccine with a MOI = 1, the 3 tumor cell lines were productively infected with measles virus vaccine, ranging from 50% of A549 cells positive for GFP to 90% of Meso13 cells (Fig. 1B). Furthermore, we observed syncytia formation for the 3 tumor cell lines (Supplementary Fig. Video S1). pDC were not permissive at MOI = 1 (Supplementary Fig. Video S2). Without a survival signal such as IL-3, the pDC died during the 72 hours of culture. Thus, we also carried out experiments in which we added IL-3 to the pDC exposed to measles virus vaccine (Fig. 1C). In the presence of IL-3, they survived during the 72 hours, but were not productively infected by measles virus vaccine. To confirm this result, we increased the MOI up to 50 in the presence of IL-3, but we still failed to detect infected pDC (Fig. 1D). However, we observed a small shift of fluorescence at MOI = 50, which was probably due to uptake of soluble GFP during the 72-hour culture, which contaminates the MV-GFP preparation, as when we used UV-irradiated MV-GFP, which is not able to replicate, we still observed this slight fluorescence shift (Supplementary Fig. S3). Finally, when the MV-GFP was incubated for 2 hours at MOI = 50 with pDC and washed, we failed to detect the small shift of fluorescence 70 hours later (Supplementary Fig. S3).
Measles virus vaccine–infected tumor cells induce maturation of pDC

We next investigated the effects of measles virus vaccine alone and measles virus vaccine–infected cells on pDC maturation (Fig. 2). In these experiments, we evaluated how measles virus vaccine infection of tumor cells in comparison with UV irradiation, another inducer of tumor cell death, affects pDC maturation. As a control for maturation, we exposed pDC to the TLR7/8 agonist, R848 (Fig. 2A and B).

We have previously shown that the measles virus vaccine–infected MPM tumor cell line, Meso13, induced maturation of monocyte-derived dendritic cells without additional adjuvants, whereas the virus alone or UV-irradiated Meso13 did not (7). We presently carried out a set of experiments on pDC to determine the effects of measles virus vaccine alone, measles virus vaccine–infected, or UV-irradiated tumor cells on pDC maturation status. We compared the effect of measles virus vaccine–infected and UV-irradiated tumor cells on the maturation status of pDC (Fig. 2). We observed maturation of pDC cocultured with measles virus vaccine–infected tumor cells, whereas UV-irradiated tumor cells failed to activate pDC. Indeed, CD83 maturation marker expression was induced by measles virus vaccine–infected cells to a similar level as that observed when the pDC were exposed to R848. We also noted an induction of the expression of the costimulation molecules, CD40 and CD86, on pDC exposed to measles virus vaccine–infected tumor cells, although this induction was low compared with the levels triggered by R848 alone.

Two studies have been reported which describe conflicting results on the ability of measles virus vaccine alone to trigger pDC maturation (30, 31). However, the study from Duhem and colleagues, reporting that measles virus vaccine activates pDC, was conducted in the presence of IL-3, a pDC survival factor (31), whereas the other study, from Schlenker and colleagues, who observed that pDC cultured with measles virus vaccine does not induce pDC maturation, was carried out without IL-3. Thus, we conducted and compared the 2 conditions and found similar results to those described by these authors. Indeed, measles virus vaccine at MOI = 1 induced pDC maturation only in the presence of IL-3 (Fig. 2). As observed for R848 alone, measles virus vaccine in the presence of IL-3-induced pDC maturation, mainly characterized by a significant increase of CD83 and, to a lesser extent, CD40 and CD86 expression. We also observed survival and maturation of pDC in the absence of IL-3 only when we exposed them to a high quantity of measles virus vaccine (MOI = 50; data not shown). At a lower viral concentration in the absence of IL-3, the pDC died.

In the last set of experiments, we tested whether measles virus vaccine infection and replication in pDC were needed to induce their activation. We exposed pDC to UV-irradiated measles virus vaccine (MV), which is unable to replicate, and we observed a similar level of maturation (CD83, CD80, and CD86 expressions) and IFN-α production as with nonirradiated measles virus vaccine (Supplementary Fig. S4A and S4C). The presence of a blocking anti-CD46–specific antibody in the culture of pDC exposed to IL-3 and measles virus vaccine did not affect maturation of pDC.
(Supplementary Fig. S4A). The same experiment was carried out with pDC exposed to measles virus vaccine–infected tumor cells. We still observed maturation and IFN-α production when measles virus vaccine–infected tumor cells were UV-irradiated before exposure to pDC (Supplementary Fig. S4B and S4C). Finally, we tested whether a CD46–specific monoclonal antibody was able to inhibit pDC maturation in response to measles virus vaccine–infected tumor cells (Supplementary Fig. S4B and S4C). We did not observe inhibition, whereas the anti-CD46 antibody completely inhibited infection of Meso13 as a control (Supplementary Fig. S4D). Altogether, these results suggest that measles virus vaccine infection and replication in pDC are not necessary for pDC activation in response to measles virus vaccine.

**pDC capture cellular components from measles virus vaccine–infected tumor cells**

Because of endo/lysosomal expression of TLR-7 and TLR-9, pDC are specialized in viral nucleic acid detection (18). These 2 receptors are the major innate receptors that activate pDC (32). Because measles virus vaccine, in the presence of IL-3 or measles virus vaccine–infected tumor cells, are able to induce pDC maturation, it is likely that the maturation stimulus is measles virus vaccine ssRNA, which activates TLR7 in the endo/lysosomal compartment. This hypothesis is strengthened by the fact that measles virus vaccine alone does not induce monocyte-derived dendritic cell maturation, as these cells do not express TLR7 in humans. This implies that some measles virus vaccine are endocytosed by pDC when they are cultured with measles virus vaccine and IL-3 or with measles virus vaccine–infected cancer cells. We then investigated whether pDC efficiently take up cellular material from measles virus vaccine–infected and UV-irradiated tumor cells (Fig. 3). Measles virus vaccine–infected and UV-irradiated M18 and A549 tumor cells were labeled with PKH67 and cocultured with pDC. We observed that pDCs efficiently take up measles virus vaccine–infected tumor cells at 37°C, whereas UV-irradiated tumor cells were less efficiently taken up (Fig 3A and B). In 2 additional experiments, we observed that the presence of the CD46 monoclonal antibody in the culture did not inhibit phagocytosis of measles virus vaccine–infected tumor cells (data not shown).

![Figure 3. Phagocytosis of measles virus vaccine–infected or UV-irradiated tumor cells by pDC. A, measles virus vaccine–infected and UV-irradiated tumor cells were stained with PKH-67 and cocultured with pDC for 18 hours at 4°C or 37°C (1 dendritic cell:1 tumor cell). Cells were stained with HLA-DR–specific mAb. Fluorescence was analyzed by flow cytometry. This experiment is representative of 4 experiments. B, scatter plot representation of the 4 phagocytosis experiments. Error bars represent the SD. C, measles virus vaccine–infected tumor cells were stained with PKH-67 (green) and cocultured with pDC for 18 hours. Cells were stained with HLA-DR–specific mAb (red). Fluorescence was analyzed by confocal microscopy.](https://clincancerres.aacrjournals.org/content/19/5/1152/F3.large.jpg)
These results were confirmed by confocal microscopy (Fig. 3C). pDC were cocultured for 18 hours with PKH-67–labeled, measles virus vaccine–infected tumor cells. The optical sections showed fluorescent fragments of measles virus vaccine–infected tumor cells inside the pDC, confirming the internalization of measles virus vaccine–infected tumor cell pieces by pDC. Interestingly, we never observed syncitia formation between pDC and tumor cells. Altogether, these results suggest that some measles virus vaccine contained in infected tumor cells could access compartments in which TLR7 is located.

Measles virus vaccine–infected tumor cells induce strong type I IFN secretion by triggering TLR7

pDCs are known to be the strongest producers of type I IFN, notably against virus, upon TLR-7 or TLR-9 activation (18). Thus, we measured IFN-\(\alpha\) production by pDC following exposure to measles virus vaccine, measles virus vaccine—infected, or UV-irradiated tumor cells by ELISA (Fig. 4A). Direct exposure to measles virus vaccine induced IFN-\(\alpha\) secretion by pDC only in the presence of IL-3, matching the cell maturation observed earlier in Fig. 2. The amount of IFN-\(\alpha\) produced in response to measles virus vaccine in the presence of IL-3 was comparable with the amount induced by R848 alone, a potent TLR7/8 agonist. Strikingly, we found high amounts of IFN-\(\alpha\) in coculture supernatants after exposure of pDC to measles virus vaccine–infected tumor cells (20–40 times more than observed in response to measles virus vaccine in the presence of IL-3 or R848 alone). These high quantities of IFN-\(\alpha\) were produced by the pDC, as tumor cells did not produce IFN-\(\alpha\) or a very low amount (pg/mL range) after measles virus vaccine infection (data not shown). UV-irradiated A549 or M18 tumor cells did not induce IFN-\(\alpha\) production by pDC. These results show that measles virus vaccine–infected tumor cells are able to trigger the production of high levels of IFN-\(\alpha\) by pDC, considerably higher than the levels produced by pDC exposed to measles virus vaccine in the presence of IL-3 or to R848 alone.

We have previously shown that, 3 days after infection of the Meso13 tumor cell line, a large amount of virus is produced, reaching \(1 \times 10^{9} \text{TCID}_{50}/\text{mL}\) corresponding to an MOI greater than 100 from a starting dose of virus of \(1 \times 10^{6} \text{TCID}_{50}/\text{mL}\), corresponding to an MOI \(= 1\) (7). It is thus likely that the huge quantity of IFN-\(\alpha\) produced by pDC in response to measles virus vaccine–infected tumor cells is the result of the intense measles virus vaccine replication in these tumor cells. To test this hypothesis, we cultured pDC in the presence of increasing MOI ranging from 1 to 50, with or without IL-3 (Fig. 4B). In the presence of IL-3, we observed that IFN-\(\alpha\) production by pDC increased with the MOI. On the contrary, pDC did not produce IFN-\(\alpha\) in the absence of IL-3, except for the highest MOI (MOI = 50).

Figure 4. Production of IFN-\(\alpha\) by pDC in response to measles virus vaccine is TLR7 dependent. A, pDC were cultured for 18 hours with IL-3, measles virus vaccine (MOI = 1), measles virus vaccine and IL-3, R848, UV-irradiated- or measles virus vaccine–infected M18 or A549 tumor cells. IFN-\(\alpha\) production was measured by ELISA in the culture supernatants. B, pDC were cultured for 18 hours with or without IL-3 and increasing quantities of measles virus vaccine. IFN-\(\alpha\) production was measured by ELISA in the culture supernatants. C, pDC were cultured for 18 hours with IL-3 and measles virus vaccine (MOI = 10), CpG-A or measles virus vaccine–infected M18, in the absence or presence of different concentrations of IRS681 (TLR7 inhibitor). IFN-\(\alpha\) production was measured by ELISA in the culture supernatants. Results were obtained from 3 independent experiments.
These results suggest that the level of IFN-α production by pDC is dependent on the quantity of measles virus vaccine and the presence of either IL-3 or other survival signals, explaining the huge quantity of IFN-α produced in response to the high titer of virus after infection of tumor cells.

Because measles virus vaccine and measles virus vaccine–infected tumor cells contain viral ssRNA, it is likely that IFN-α production by pDC is mainly due to the triggering of TLR-7. Thus, we carried out an inhibition of TLR7. We used specific IRS that inhibit IFN-α expression mediated by TLR-7 (IRS661; ref. 33). We showed that IFN-α production by pDC cultured in the presence of measles virus vaccine and IL-3 was inhibited when we added the IRS661 (Fig. 4C). We also observed a similar IFN-α inhibition when IRS661 was added to pDC exposed to measles virus vaccine–infected tumor cells. As a control, we showed that IRS661 did not
inhibit the CpG-A–induced IFN-α production by pDC, which is TLR9-dependent. Altogether, these results show that IFN-α production induced by measles virus vaccine or measles virus vaccine–infected cells is TLR7 dependent.

**pDC are able to cross-present a tumor-associated antigen from measles virus vaccine–infected tumor cells**

The capacity of human DC to cross-present viral antigens has been reported (20–22), but cross-presentation of tumor-associated antigens (TAA) has not yet been described. We wondered whether human pDC exposed to measles virus vaccine–infected tumor cells would be able to cross-present a human TAA spontaneously expressed by tumor cells. We showed by real-time PCR that the HLA-A*0201-positive M18 melanoma cell line expresses the cancer testis antigen, NYESO-1, whereas the A549 lung adenocarcinoma cell line does not (Fig. 5A).

To determine whether HLA-A*0201-positive pDC are able to cross-present this TAA after exposure to an measles virus vaccine–infected or UV-irradiated HLA-A*0201-positive NYESO-1-positive M18 tumor cell line, we used the CD8+ T-cell clone, M117.167, which is specific for HLA-A*0201/NYESO-1(157–165) complexes (Fig. S5B–D). A schematic of this experiment is shown in Supplementary Fig. S5. The M117.167 T-cell clone did not produce IFN-γ, alone or in the presence of IL-3 pDC but was activated in the presence of pDC pulsed with NYESO-1(157–165) peptides (Fig. 5B). The clone was activated as soon as 0.1 μmol/L peptide was loaded onto pDC (16.3% IFN-γ+ cells) and was more intensely activated by pDC pulsed with 1 μmol/L peptide (77.5%). In the presence of pDC cultured with measles virus vaccine–infected M18 tumor cells, 11.5% of the clone population was activated, whereas the clone did not produce IFN-γ in response to pDC cultured with UV-irradiated M18 tumor cells (Fig. 5B). In response to pDC cocultured with measles virus vaccine–infected M18, the clone had an IFN-γ production profile comparable with that observed in response to pDC pulsed with 0.1 μmol/L NYESO-1(157–165) peptide.

As a control, we failed to detect activation of the M117.167 T-cell clone in response to measles virus vaccine–infected or UV-irradiated M18 tumor cells alone (Fig. 5C). This result was expected, as the M18 tumor cell line is HLA-A*0201-positive, thus unable to directly present NYESO-1 (157–165) peptide to the clone. This shows that IFN-γ production by the clone in response to HLA-A*0201-positive pDC cocultured with measles virus vaccine–infected M18 tumor cells is due to cross-presentation. We also did not observe IFN-γ production in response to pDC cocultured with measles virus vaccine–infected NYESO-1-positive A549 tumor cells. In this representative experiment, the clone produced IFN-γ in response to pDC cocultured with measles virus vaccine–infected M18 (6.5% IFN-γ+ cells), a production rate close to the one observed in response to pDC pulsed with 0.1 μmol/L NYESO-1(157–165) peptide (10.8% IFN-γ+ cells). In a final set of experiments, we compared NYESO-1 cross-presentation by pDC with cross-presentation by Mo-DC (Supplementary Fig. S6). We found a similar level of cross-presentation between the 2 types of dendritic cells when they were cocultured with measles virus vaccine–infected M18 tumor cells, whereas no cross-presentation was observed in response to pDC or Mo-DC cocultured with UV-irradiated M18.

Altogether, our results show that pDC are able to cross-present tumor antigen such as NYESO-1 from measles virus vaccine–infected tumor cells, but not from UV-irradiated ones. Thus, measles virus vaccine–based antitumor virotherapy should be able to hire pDC in the antitumor immune response by activating their ability to produce high quantities of IFN-α and to cross-present TAA from measles virus vaccine–infected tumor cells to tumor-specific CD8+ T lymphocytes.

**Discussion**

In this study, we characterized, *in vitro*, the consequences of measles virus vaccine–based antitumor virotherapy on human pDC functions. First, we showed that pDC are not sensitive to measles virus vaccine infection despite expression of CD46. However, they are able to detect the virus by producing IFN-α in response to high virus quantity in the absence of a survival signal, and to low virus quantity when a survival signal, such as IL-3, is added to the culture. Second, when the pDC were cocultured with measles virus vaccine–infected tumor cells, they underwent a maturation characterized by the induction of CD83 expression and strong production of IFN-γ, with a slightly increased expression of costimulatory molecules. Conversely, the pDC cocultured with UV-irradiated tumor cells retained an immature phenotype similar to that observed when they were cocultured with IL-3 alone. We then identified TLR7 as the pDC receptor responsible for their activation, probably due to the presence of single-stranded viral RNA in the endocytic compartment of pDC following internalization of measles virus vaccine–infected tumor cell fragments. Finally, using an HLA-A*0201/NYESO-1 (157–165)–specific CD8+ T-cell clone, we showed that HLA-A*0201-positive pDC were able to cross-present this TAA from NYESO-1-positive HLA-A*0201-positive measles virus vaccine–infected tumor cells but not from UV-irradiated ones. This is the first time, to our knowledge, that the capacity of human pDC to cross-present a TAA from dead tumor cells to CD8+ T cells has been shown. Altogether, these results suggest that measles virus vaccine–based antitumor virotherapy, in addition to its direct lysis of infected tumor cells, is able to recruit pDC in the antitumor immune response, to activate their ability to produce high levels of type I IFN and to cross-present TAA.

In the first part of our work, we showed that human pDC exposed *in vitro* to measles virus vaccine at an MOI = 1 did not undergo maturation without IL-3. In this condition, with no survival signal, pDC undergo apoptosis and fail to acquire measles virus vaccine in the endosomal compartment to engage in a maturation process by the ligation of viral ssRNA to TLR7. When pDC were exposed to measles virus vaccine in the presence of IL-3, they survived and...
maturation was observed (low IFN-α production and induction of CD83 expression). We observed the activation of pDC by measles virus vaccine in the absence of IL-3, only when we used a high quantity of measles virus vaccine (MOI = 50). At this high measles virus vaccine concentration, we think that enough measles virus vaccine reached the endocytic compartment of pDC to provide a survival/maturation signal, before their apoptosis program was engaged. Thus, when pDC are exposed to measles virus vaccine in the presence of IL-3, the pDC survive and measles virus vaccine is internalized and allows triggering of TLR7 by the viral ssRNA. When pDC are exposed to measles virus vaccine in the absence of IL-3, they undergo apoptosis unless enough measles virus vaccine reaches the endocytic compartment to activate and mature them. Our results explain the contradictory reports in the literature, due to differences in experimental settings. Indeed, we obtained similar results to Schlender and colleagues, who reported that a low quantity of measles virus vaccine Schwarz failed to induce IFN-α by pDC cultured in the absence of IL-3 (30), and to Duhen and colleagues who claimed that measles virus vaccine Schwarz induces high quantities of IFN-α production by pDC in the presence of IL-3 (31). However, our study does not support the claim that measles virus vaccine Schwarz inhibits IFN-α production by pDC (30), as pDC produce IFN-α in the presence of IL-3. Finally, both groups described staining of pDC by a monoclonal antibody to measles virus vaccine hemagglutinin (H), but interpreted the result differently. One group claimed that pDC were infected and amplified the virus (30), whereas the other group concluded that, despite the H protein staining on pDC, measles virus vaccine replication was low. Our results support this latter conclusion, as we did not observe productive infection using MV-eGFP, even at high MOI, in the absence nor presence of IL-3.

We also showed that, in the presence of measles virus vaccine or measles virus vaccine–infected tumor cells, pDC undergo maturation characterized by the upregulation of CD83 molecule expression at the cell surface. In the presence of measles virus vaccine or measles virus vaccine–infected tumor cells, the pDC produce higher quantities of IFN-α in response to high viral load than pDC stimulated with R848 alone. However, these cells do not express as much of the CD40 and CD86 costimulatory molecules. Thus, this maturation phenotype resembles the maturation phenotype induced by HIV infection (34, 35), which activates pDC by the TLR7, as does measles virus vaccine (36). Indeed, it is now clear that, depending on the nature of the TLR agonist used, 2 main pathways of activation can be triggered in human pDC. This dichotomy was first reported by Kerkmann and colleagues, who showed that 2 TLR9 agonists, CpG-A and CpG-B, activate pDC maturation using 2 different pathways (37). More recently, the same dichotomy has been observed for TLR7 agonists (35). Indeed, HIV behaves like CpG-A by triggering TLR7 and the IRF7 signaling pathway in the early endosome of pDC, and by inducing strong production of IFN-α. Our results show that the maturation induced by MV + IL-3 or measles virus vaccine–infected cells is similar to the activation induced by HIV, suggesting an early endosomal triggering of TLR7 by measles virus vaccine ssRNA. This early endosome activation pathway is compatible with antigen cross-presentation expressed by virus-infected cells, as cross-presentation of viral antigens from infected cells has been shown (21) and cross-presentation of the TAA from measles virus vaccine–infected cells, in our present study. Conversely, Schnurr and colleagues reported that, in vitro, pDC, contrary to myeloid dendritic cells, were not able to cross-present a TAA from a full-length protein alone or as an immune complex form (38). However, these authors used a soluble protein and did not use NYESO-1–expressing tumor cells as the antigen source. In vivo, antigen cross-presentation by pDC is also controversial. Salio and colleagues reported that murine pDC stimulated by CpG are not able to cross-present antigens, whereas they can mount a T-cell response against endogenous antigens (39). Mouries and colleagues showed, in vivo and in vitro, also in a murine model, that soluble OVA protein and TLR agonists (CpG or R848) activate pDC to cross-prime OVA to specific CD8+ T cells (23). Similarly, presentation and cross-presentation of soluble OVA peptide or whole protein, following TLR9 stimulation by CpG or by infection with influenza virus containing OVA epitopes, was confirmed recently, in vitro, by Kool and colleagues (40). Finally, Liu and colleagues reported that intratumoral injection of CpG-A–stimulated pDC to mice bearing B16 melanoma induced a tumor antigen cross-primer, but this cross-priming was conducted by CD11c+ dendritic cells, not by pDC (25). We have shown here that, in vitro, human pDC exposed to measles virus vaccine–infected tumor cells are able to cross-present NYESO-1 to a CD8+ T-cell clone specific for this TAA. We showed that measles virus vaccine–infected tumor cells undergo cell death and are then phagocytosed by pDC. These measles virus vaccine–infected cells are capable of activating pDC without the addition of adjuvants or TLR agonists. It remains to be determined whether this cross-presentation would result in cross-primer in vivo as measles virus vaccine activates pDC by the early endosomal pathway, which is thought to be more implicated in IFN-α production than in T-cell stimulatory capacity (41).

The efficiency of measles virus vaccine–based antitumor virotherapy has been shown in vivo in different models of human tumor xenografts in immunodeficient mice (8–11). The first clinical trials of measles virus vaccine–based virotherapy have shown encouraging results (13, 14). The efficiency of measles virus vaccine–based virotherapy is likely due to the lysis of tumor cells by the virus. However, a part of its efficiency may also be due to the capacity of measles virus vaccine–infected tumor cells to activate cells of the immune system, notably pDC. Indeed, activation of pDC by TLR agonist in tumor-bearing mice has been shown to induce an antitumor immune response and tumor regression (24, 25, 42). Liu and colleagues showed that murine pDC stimulated by a TLR9 agonist induced natural killer (NK) cell activation and recruitment to the tumor, triggering tumor antigen cross-presentation by CD11c+
dendritic cells (25). Drobits and colleagues showed that topical treatment of melanoma tumors in mice with the TLR7 agonist, imiquimod, induced activation and recruitment of pDC into the tumor and caused tumor regression (24). They showed that pDC acquire a cytotoxic activity against tumor cells by secreting TRAIL and granzyme B, in an IFNAR1-dependent mechanism. IFN-α secretion by pDC not only induces an antitumor cytotoxic activity on pDC by an autocrine loop but can also act directly on tumor cells to induce apoptosis (43). Type I IFN also plays a role in the NK activation and is required in a mouse model of NK-cell–dependent tumor rejection (44). Finally, these NK cells probably also participate in the initiation of the antitumor response by stimulating myeloid dendritic cell, as in IFNAR1- and STAT1-deficient mice the antitumor T-cell response failed to develop (45, 46). Thus, our study shows that measles virus–infected tumor cells induce a high quantity of IFN-α by pDC, which may be favorable for the development of multicell subsets involved in an antitumor immune response. Furthermore, other oncolytic viruses known to activate pDC are being used in clinical trials of antitumor virotherapy, such as vaccinia (47), herpes simplex virus (48) and adenovirus (49). Tumor cells infected by these viruses may also be able to induce IFN-α production and tumor antigen cross-presentation by pDC.

Measles virus vaccine–based antitumor virotherapy is a promising approach for treating cancer through the oncolytic activity of the virus. Furthermore, we show here that measles virus–infected tumor cells activate the maturation and tumor antigen cross-presentation capacities of human pDC. Thus, measles virus vaccine–based antitumor virotherapy may represent an interesting approach to the recruitment of pDC in the antitumor immune response.

References


Measles Virus Vaccine–Infected Tumor Cells Induce Tumor Antigen Cross-Presentation by Human Plasmacytoid Dendritic Cells

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