Effects of Herpes Simplex Virus Amplicon Transduction on Murine Dendritic Cells

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Abstract

The herpes simplex virus (HSV)-based amplicon is a versatile vaccine platform that has been preclinically vetted as a gene-based immunotherapeutic for cancer, HIV, and neurodegenerative disorders. Although it is well known that injection of dendritic cells (DCs) transduced *ex vivo* with helper virus-free HSV amplicon vectors expressing disease-relevant antigens induces antigen-specific immune responses, the cellular receptor(s) by which the amplicon virion gains entry into DCs, as well as the effects that viral vector transduction impinges on the physiological status of these cells, is less understood. Herein, we examine the effects of amplicon transduction on mouse bone marrow-derived DCs. We demonstrate that HSV-1 cellular receptors HveC and HveA are expressed on the cell surface of murine DCs, and that HSV amplicons transduce DCs at high efficiency (>90%) with minimal effects on cell viability. Transduction of dendritic cells with amplicons induces a transient DC maturation phenotype as represented by self-limited upregulation of MHCII and CD11c markers. Mature DCs are less sensitive to HSV amplicon transduction than immature DCs regarding DC-related surface marker maintenance. From this and our previous work, we conclude that HSV amplicons transduce DCs efficiently, but impart differential and transient physiological effects on mature and immature DC pools, which will facilitate fine-tuning of this vaccination platform and further exploit its potential in immunotherapy.

Introduction

DENDRITIC CELLS (DCs) represent the most potent antigen-presenting cells of the immune system, with their ability to initiate and regulate adaptive immune responses (Fajardo-Moser *et al.*, 2008). DCs are derived from hematopoietic progenitor cells, which have the potential to differentiate into DCs, osteoclasts, and macrophages (Ziegler-Heitbrock, 2000; Taylor and Gordon, 2003; Gordon and Taylor, 2005; Alnaeeli *et al.*, 2007). The functional plasticity of DCs is determined by their proximal environment. Before encountering antigens that arise from an infection or foreign body, DC precursors patrol peripheral tissues and lymphoid organs (Caux *et al.*, 2000). In the absence of inflammatory responses, DCs remain in an immature state (iDCs) with strong phagocytic activity. At the onset of infection, DCs mature in response to host-derived

inflammatory molecules such as CD40 ligand (CD40L), tumor necrosis factor (TNF)- α , interleukin (IL)-1, IL-6, and interferon (IFN)- α . Mature dendritic cells (mDCs) are characterized by reduced phagocytic activity, accelerated homing and migration to lymphoid tissues, enhanced T cell activation, and growth of cytoplasmic dendrites. mDCs are able to prime T cells in secondary lymphoid organs, which results in their differentiation into helper type 1 (Th1), Th2, or Th17 cells (Moser and Murphy, 2000; Guernonprez *et al.*, 2002; de Jong *et al.*, 2005; Mahnke and Enk, 2005; Galli *et al.*, 2008). Presentation of antigen by iDCs leads to tolerance, whereas antigen-loaded mDCs are geared toward the launching of antigen-specific immunity (Dubsky *et al.*, 2005).

The critical role(s) DCs play in polarizing T cells and determining the outcome of immune responses make DCs a major focus for the development of vaccination strategies.

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With their strong capability in antigen presentation, DCs have been employed as natural potent adjuvants for immunization (Mayordomo *et al.*, 1997), pulsed with target peptide(s) (Inaba *et al.*, 1990; Celluzzi *et al.*, 1996), and transduced with various viral vector platforms to efficiently present antigens of interest (Takayama *et al.*, 1999; Di Nicola *et al.*, 2003; Koya *et al.*, 2003, 2004; Timares *et al.*, 2004; He *et al.*, 2005). Many studies have shown that engineered DCs effectively deliver antigens and/or cytokines of interest to modulate the type and intensity of immune response (Condon *et al.*, 1996; Figdor *et al.*, 2004; Moll, 2004; Iwashita *et al.*, 2005). The immunotherapeutic potential of antigen-pulsed DCs for the treatment of cancer, HIV, and neuronal diseases has been confirmed in a number of experimental animal models (Mayordomo *et al.*, 1995; Celluzzi and Falo, 1998; Liu *et al.*, 2001; Zhang *et al.*, 2002; Buchsel and DeMeyer, 2006).

To date, several viral vector systems including adenovirus, lentivirus, vaccinia virus, as well as herpes simplex virus (HSV)-derived amplicons have been tested for gene delivery to DCs (Takayama *et al.*, 1999; Willis *et al.*, 2001; Di Nicola *et al.*, 2003; Koya *et al.*, 2003, 2004; Timares *et al.*, 2004; He *et al.*, 2005). The HSV amplicon possesses a number of advantages over other gene delivery platforms. First, the amplicon is not a live virus (as are vaccinia, canarypox, etc.) and, therefore, has an inherently safer *in vivo* profile. Second, compared with DNA delivery systems or most virus-based vectors, expression is directed from multiple episomal copies within each transduced cell, and the genome is maintained in non-dividing cells such as antigen-presenting cells (APCs). Third, the transgene size limit is larger (9130 kb) (Wade-Martins *et al.*, 1999, 2001, 2003) than that of many other viral vectors, affording an opportunity to coexpress factors with known immunomodulating activity. Fourth, the lack of encoded viral genes avoids the effects that wild-type herpesviruses typically use to evade the immune system, such as prolonged down-regulation of MHC expression and antigen processing, and inhibition of dendritic cell maturation (Salio *et al.*, 1999). Most importantly, HSV-1 amplicon-transduced DCs were able to express introduced antigens specifically, which led to induction of potent antitumor immunity in vaccinated mice (Willis *et al.*, 2001).

In the present study, we sought to identify the cellular receptor of HSV amplicon on DCs and to determine the effects that HSV amplicon transduction potentially imparts to phenotypic markers expressed on the surface of murine bone marrow-derived DCs. We examined surface expression of two HSV-1 cellular receptors, HveC and HveA, and monitored the dynamic changes in DC surface markers before and after HSV amplicon transduction.

Materials and Methods

Mice

Eight-week-old female C57BL/6 (B6) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Animals were maintained in sterile microisolator cages under pathogen-free conditions in accordance with ethics guidelines for the care of laboratory animals at the University of Rochester. All animal procedures were performed in compliance with guidelines established by the University Committee of Animal Resources at the University of Rochester (Rochester, NY).

Reagents

All antibodies used for flow cytometric analysis were purchased from BD Biosciences (San Jose, CA), except for CK6 and R140. Anti-HveC antibody CK6 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-HveA antibody R140 was provided by G.H. Cohen and R.J. Eisenberg (University of Pennsylvania, Philadelphia, PA).

Flow cytometric analysis

Cells were first blocked with the Fc_γR blocker 2.4G2 in FACS buffer (2% fetal calf serum [FCS] and 0.1% [w/v] NaN₃ in 1× phosphate-buffered saline [PBS]) for 15 min, followed by incubation for 20 min on ice with monoclonal antibody (mAb) conjugated with various dyes, and washed thoroughly by PBS. Data were acquired with a FACSCalibur flow cytometer (BD Biosciences), and analyzed with CellQuest software version 3.5.1 (BD Biosciences).

Isolation and culture of DCs

The protocols for DC isolation and culturing were obtained from the laboratory of A. Livingstone (University of Rochester). This protocol followed mainly the protocols published by Lutz and colleagues (1999) with minor modifications. Briefly, DC precursors were isolated from bone marrow from femurs and tibias of B6 mice. An aliquot of 2×10^6 of cells was subsequently resuspended in 10 ml of R7 medium (RPMI supplemented with 7% FCS, 2 mM glutamine, penicillin [50 IU/ml], streptomycin [50 µg/ml], and 50 µM 2-mercaptoethanol) in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF, 20 ng/ml) and cultured in a Falcon 1005 plate (BD Biosciences). On day 3, 10 ml of fresh R7 medium was added, and on day 6, 10 ml of old medium was replaced with the same volume of fresh R7 medium. On day 8, cells were transferred to a Falcon 3003 cell culture plate with lower GM-CSF concentration (5 ng/ml). On day 9, cells were harvested and used for HSV amplicon transduction and flow cytometric analysis. The percentage of DCs present in the culture was determined by the coexpression of CD11c and MHCII surface markers. mDCs were generated from day 8 iDCs by overnight incubation (~16 hr) with lipopolysaccharide (LPS, 100 ng/ml; Sigma-Aldrich, St. Louis, MO). LPS-treated DCs were washed thoroughly three times with Hanks' balanced salt solution (HBSS) before use.

HSV amplicon vector packaging

An HSV amplicon vector expressing enhanced green fluorescent protein (HSV-eGFP) and an empty control vector (HSV-PrPuc) were separately packaged and titered, using helper virus-free methodologies established in our laboratory and published previously (Bowers *et al.*, 2000, 2001).

DC transduction

For HSV amplicon transduction, DCs were collected on day 9 and resuspended at 4×10^6 cells/ml in RPMI medium. HSV amplicon vector particles expressing enhanced green fluorescent protein (HSV-eGFP) or empty vector (HSV-PrPuc) were added to DCs at various multiplicities of infection (MOIs) and were incubated for 2 hr at 37°C within a sterile

FIG. 1. Expression levels of nectin-1 and HveA on bone marrow-derived dendritic cells (DCs). Bone marrow (BM) samples were isolated from wild-type B6 mice and cultured as described in Materials and Methods. Cells were harvested and analyzed by flow cytometric analysis on day 9, using anti-nectin-1 antibody (CK6) or anti-HveA antibody (R140) combined with phycoerythrin (PE)-conjugated CD11c and allophycocyanin (APC)-conjugated MHCII antibodies. Propidium iodide (PI) was added to cells before flow cytometric analysis. (A) Dot-plot analysis of total cells. The R1 gate represents live cells that were PI negative. FSC, forward scatter. (B) Quadrant analysis of live cells. Live cells (R1 gated) were further classified into four different distinct cell populations (designated as R2 to R5) according to their surface expression of CD11c and MHCII markers. Expression levels of HveC or nectin-1 (C) or HveA (D) were analyzed by overlaid histogram analysis. Expression levels of HveC in live CD11c⁺MHCII⁺ double-positive cells (R1 and R3 gated) and in the CD11c⁺MHCII⁻ population (R1 and R5 gated) are shown by solid green and open pink curves, respectively. The expression level of HveA is shown in blue (right). The black curve represented staining of the isotype control.

FACS tube. DCs were then washed three times with HBSS and resuspended at 10^7 cells/ml for phenotype characterization and subsequent analysis. Three independent experiments were performed in triplicate for each transduction condition per time point.

Results

HSV-1 receptors HveA and HveC were expressed on the cell surface of murine bone marrow-derived DCs. Herpes simplex virus type 1 (HSV-1) is a naturally neurotropic DNA virus proficient in establishing latent infection within neurons, but also possesses the ability to infect a wide range of cell/tissue types. The entry of HSV type 1 virions, including HSV-derived amplicon vectors, occurs via a two-step process: First, glycoproteins B (gB) and C (gC) embedded within the virion envelope bind ubiquitous heparan sulfate proteoglycans found on a target cell's surface. Second, this interaction leads to a conformational change in HSV envelope glycoprotein D and subsequent binding to cellular receptors responsible for virion docking and uptake, including the herpesvirus entry mediator A (HveA; formerly HVEM) and nectin-1 (HveC) (Johnson *et al.*, 1988). We initially sought to determine whether these two HSV receptors were expressed on bone marrow-derived dendritic cells, and if so, whether differences in expression profiles

exist within specific cellular populations. BM-derived cells were generated according to protocols published by Lutz and colleagues (1999) and used for all our experiments. On the basis of the surface expression of two DC-specific markers, CD11c and MHCII, live (PI negative, R1 gated) BM-derived cells were categorized into four groups (Fig. 1A and B: R2, CD11c⁻MHCII⁺; R3, CD11c⁺MHCII⁺; R4, CD11c⁻MHCII⁻; R5, CD11c⁺MHCII⁻). Cells localized to the R3 gate (CD11c⁺MHCII⁺) represent the BM-derived DC population.

Antibodies CK6 and R140 were used to assess the dendritic cell surface expression of HveC and HveA, respectively. CK6 is a monoclonal antibody that recognizes the ectodomain of human HveC (Krummenacher *et al.*, 2000), whereas R140 is a rabbit polyclonal antibody raised specifically against HveA (Terry-Allison *et al.*, 1998). CK6 has been shown to specifically recognize the mouse form of HveC by Western blotting (Lim *et al.*, 2008), and, moreover, this antibody has been demonstrated to detect HveC on the cell surfaces of several cell lines and to block the cellular entry of HSV particles (Krummenacher *et al.*, 2000). Interestingly, the expression of HveC was detected only on cells comprising the R3 population (CD11c⁺MHCII⁺, double positive), but not others (R2 and R4 [data not shown]), and R5), indicating that the expression of HveC is DC specific (Fig. 1C). In contrast, expression of the other HSV receptor, HveA, on DCs (R3) was more modest (Fig. 1D),

FIG. 2. HSV amplicon transduces BM-derived DCs efficiently. Day 9 BM-derived DCs were transduced with HSVeGFP at a multiplicity of infection (MOI) of 0 (A), 0.5 (B), or 1.0 (C). Cells were subsequently resuspended in R7 medium in the presence of rGM-CSF for 10 hr to allow expression of eGFP. The histogram analysis portrays the percentage of eGFP⁺ cells in the total cell population.

although the expression level of HveA on CD11c⁺MHCII⁺ cells was still higher than that expressed on the other BM-derived cell populations (R2 and R4 [data not shown], and R5).

HSV amplicons transduce all BM-derived cell subsets efficiently. Although HSV amplicons are known to transduce many varieties of cell types (Oehmig *et al.*, 2004a,b), higher expression of the HSV entry receptors HveC and HveA on BM-derived DCs (Fig. 1, R3) raised the possibility that the HSV amplicon may preferentially transduce this particular population of BM-derived cells. To examine this possibility, we transduced all BM-derived cells at various multiplicities of infection (MOIs of 0, 0.5, and 1) with HSVeGFP and assessed the efficiency of transduction off each cell subset as represented by the percentage of eGFP⁺ cells. HSVeGFP is an HSV amplicon that expresses the enhanced green fluorescent protein (eGFP) reporter gene and was previously constructed in our laboratory (Detrait *et al.*, 2002).

As shown in Fig. 2, HSVeGFP transduced BM-derived DCs with high efficiency, a result that could be predicted given the high-level expression of the two major HSV-1 receptors by

this cell population. A slight enhancement (82–91%) was observed as the MOI (as determined by titrating on NIH/3T3 cells) was increased from 0.5 to 1. Subsequently, we examined whether HSVeGFP transduction was limited to the CD11c⁺MHCII⁺ DC subset (R3) or could be detected in the other

FIG. 3. HSV amplicons are able to transduce all subsets of BM-derived cells. Day 9 BM-derived DCs were transduced with HSVeGFP at an MOI of 0.5 for 2 hr and cultured in R7 medium supplemented with rGM-CSF to allow expression of eGFP. Cells were then washed extensively, blocked, and stained with CD11c-PE/MHCII-APC. PI was added to the cells before flow cytometric analysis. BM-derived DCs were classified into four groups, R2 (A), R3 (B), R4 (C), and R5 (D), according to their surface expression of CD11c and MHCII as defined in Fig. 1. Solid green curves represent the expression level of eGFP in the corresponding untransduced cell population (background), and pink curves represent the expression level of eGFP on HSVeGFP-transduced cells.

FIG. 4. mDCs (+LPS) and iDCs (–LPS) respond differently to HSV amplicon transduction. BM-derived DCs were generated as stated in the caption to Fig. 1. On day 8, half the cells were treated with LPS for 16 hr and used as mDCs, and half the cells were left untreated and used as iDCs. Before HSV amplicon transduction, the phenotypes of iDCs and mDCs were examined by flow cytometry. (A and B) Distribution of BM-derived cell populations with LPS (A) or without LPS (B) before HSV amplicon transduction. Quadrant analysis was gated on live cells (PI negative). On day 9, cells were subject to amplicon transduction at various MOIs (0, 0.5, 1.0, and 5.0), followed by phenotype characterization by flow cytometric analysis. (C) Dynamic changes in R3 and R5 populations ($CD11c^+MHCII^+$ and $CD11c^+MHCII^-$, respectively, as defined in Fig. 1) after amplicon transduction (MOI, 0 to 5). Solid columns and open columns represent the percentage of R3 and R5 cells within total live cells, respectively. Error bars indicate the standard deviation and an asterisk (*) indicates $p < 0.05$ as determined by paired Student *t* test. (D) Percentage of dead cells (PI positive) in LPS-treated and untreated BM-derived cells after HSV amplicon transduction. Error bars indicate the standard deviation.

BM-derived cell populations (R2, R4, and R5 as defined in Fig. 1). Figure 3 depicts the histogram analysis of eGFP expression in cell populations R2 to R5. This result suggested that HSVeGFP could transduce all BM-derived cells readily regardless of their surface expression of CD11c and MHCII markers. This observation, together with the specific expression of HSV receptors HveA and HveC only on the $CD11c^+MHCII^+$ DC subset (R3), suggested that several redundant cell surface receptors might be involved in mediating HSV amplicon entry into cells besides HveA and HveC. It is noteworthy that the $CD11c^-MHCII^+$ subset (Fig. 3A) showed the highest amplicon transduction efficiency as represented by the highest mean fluorescence intensity (MFI) of eGFP.

HSV amplicon transduction leads to dynamic, albeit transient, changes in DC surface marker expression profiles. iDCs and mDCs are distinct from one another with respect to several biochemical, phenotypical, and functional features. iDCs express limited levels of empty but peptide-receptive MHCII molecules on their cell surface and are adept at capturing antigen. mDCs, by contrast, express high levels of peptide-loaded MHCII on their cell surface, but lose their phagocytic ability to capture new antigens. We sought to address two important questions regarding the maturation status of DCs: (1) Do iDCs and mDCs respond differently to HSV amplicon transduction? and (2) Does HSV amplicon transduction serve as a maturation signal for DCs?

We followed the protocol previously published by Lutz and colleagues (1999) to generate BM-derived DCs. Using this

method, the majority of $CD11c^+MHCII^+$ double-positive cells are considered phenotypically iDCs. These iDCs require further inflammatory stimuli such as bacterial lipopolysaccharide (LPS) for further maturation. On day 8, one-half of the BM-derived cells were treated with LPS overnight to generate mDCs, whereas the remaining cells were left untreated and considered iDCs. Both populations were transduced with HSV amplicon at various MOIs (0, 0.5, 1, and 5) on day 9. As shown in Fig. 4A, incubation of BM-derived cells with LPS significantly drove cells toward a mature phenotype represented by an increase in the percentage of $CD11c^+MHCII^+$ cells as well as up-regulation of MHCII and CD11c surface expression. This observation demonstrated that our preparation of BM-derived cells remained immature without receiving any exogenous stimuli during culturing (Fig. 4B). These cells were used as iDCs (–LPS) and mDCs (+LPS) in our experiment. Their responses to HSV amplicon transduction are compared and summarized in Fig. 4C and D.

For mDCs (+LPS), the percentage of R3 and R5 subsets among total cells remained constant even when the HSV titer was increased from 0 to 5 (Fig. 4C, left), suggesting that HSV amplicon transduction did not induce significant physiological changes in mDCs. In contrast, HSV amplicon transduction profoundly altered the distribution of R3 and R5 subsets if cells remained immature (–LPS) (Fig. 4C, right). When the MOI was increased from 0 to 5, the percentage of mDC population R3 decreased from 39 to 19%. Conversely, there was a concomitant increase in the iDC population (R5) as the MOI

increased (Fig. 4C, the rightmost open column). This result suggested that higher numbers of HSV amplicon particles might be toxic to BM-derived iDCs and inhibit surface MHCII expression. It is possible that binding of HSV amplicon particles to cells promotes internalization of cell surface MHCII molecules, thus resulting in the decrease in R3 population and concomitant increase in R5 population (Fig. 4C, right). Notably, we found that the surface expression of CD11c, unlike MHCII, is inert to HSV amplicon transduction if BM-derived cells remained immature, as the total percentage of CD11c⁺ cells (R3 plus R5) remained invariable regardless of HSV amplicon particle numbers (Fig. 4C and Table 1).

The enhanced sensitivity of iDCs to HSV amplicon transduction begged the question as to whether the gradual loss of surface expression of MHCII surface markers on iDCs correlated with higher lethality on these cells. To this end, we determined the cell viability of LPS-treated and untreated cells at progressively higher MOIs (Fig. 4D). In the absence of amplicon transduction, no statistically significant differences in viability were detected in LPS-treated cells versus untreated populations. Moreover, the percentage of dead cells did not increase as the amplicon MOI was raised (Fig. 4D), indicating that HSV amplicon transduction imparted more profound effects on MHCII surface expression on iDCs (Fig. 4C), a phenomenon that is independent of any apparent toxicity induced by amplicon application.

HSV-transduced DCs undergo a dynamic and rapid physical change. To determine whether the effect of HSV amplicon transduction on BM-derived DCs is transient, we monitored the expression of DC surface markers as a function of time after amplicon transduction. Addressing this question would help to establish the optimal time at which to administer HSV-transduced DCs to animals posttransductionally. Untreated iDCs were used for this experiment, because HSV amplicon transduction induced a significant change in iDCs, but not in LPS-treated mDCs (Fig. 4C). In addition, an MOI of 2 was chosen for this experiment, because we believed that this condition is within the range of efficient transduction and moderate toxicity (Fig. 4D).

We illustrate the dynamic changes in CD11c and MHCII markers after HSV amplicon transduction in Fig. 5. HSV amplicon transduction induced an immediate, sharp decrease (74 to 63%) in the R3 CD11c⁺MHCII⁺ subset (Fig. 5A and B), a finding consistent with our previous observation (Fig. 4C). Two hours after transduction, the mean fluorescence intensity (MFI) of surface MHCII marker staining was up-regulated and more than doubled (Fig. 5I), suggesting HSV amplicon transduction drove iDCs toward a more mature phenotype. During these 2 hr, the immature R5 cell subset (CD11c⁺MHCII⁻) originally observed at the prior time point disappeared completely (Fig. 5B and C). We surmised that BM-derived iDCs underwent a rapid enhancement of surface marker expression reminiscent of maturation within the first 4 hr after amplicon transduction. Both MHCII and CD11c staining intensities began to decline 6 hr posttransduction (Fig. 5K and Q), and continued to drop at the subsequent time point. Cells had lost their surface expression of CD11c marker significantly by 8 hr after transduction (Fig. 5R). As a control, we monitored the expression of MHCII and CD11c on nontransduced cell counterparts at identical time points (data not shown). It is clear that the rapid dynamic change in surface CD11c and MHCII was caused by HSV amplicon transduction.

TABLE 1. CORRELATION OF HERPES SIMPLEX VIRUS AMPLICON MULTIPLICITY OF INFECTION AND PERCENTAGE OF MATURE (R3) AND IMMATURE (R5) CELL SUBSETS IN MURINE BONE MARROW-DERIVED CELLS

Percentage of total cells	MOI			
	0	0.5	1	5
R3 (CD11c ⁺ MHCII ⁺)	39	34	34	19
R5 (CD11c ⁺ MHCII ⁻)	28	31	33	45
Sum (R3 + R5)	67	65	67	64

Abbreviation: MOI, multiplicity of infection.

In addition to CD11c and MHCII, two characteristic markers of DCs, we examined whether MHCI, the other antigen-presenting molecule, and CD80, an important costimulatory molecule of DCs, were also affected by HSV amplicon transduction. Figure 5S summarizes the dynamic change in these molecules after amplicon transduction. The expression levels of each surface marker were compared with those of its nontransduced cell counterpart. Our results indicated that HSV amplicon transduction caused an upregulation of MHCI, MHCII, and CD11c, and reached the maximum by 2 hr after transduction. Among the four molecules examined, the extent of MHCII elevation was the highest (~2.5×-fold), whereas the level of surface CD80 was relatively unaltered. Interestingly, the expression level of CD11c decreased dramatically 8 hr after transduction, to a level lower than that observed before HSV amplicon transduction.

Discussion

We previously showed that the HSV amplicon is an effective gene transfer and vaccine platform for the preclinical evaluation of therapeutic actions in many mouse models of neurological and oncologic diseases (Geschwind *et al.*, 1996; Carew *et al.*, 2001; Zager *et al.*, 2001; Bennett *et al.*, 2002; Bowers *et al.*, 2002, 2005; Delman *et al.*, 2002; Sortwell *et al.*, 2007). These prior results led us to develop an immunotherapeutic approach by combining the HSV amplicon vaccine platform with the most potent antigen-presenting cells, dendritic cells. Along with our colleagues, we have demonstrated that HSV amplicon-transduced DCs induce antigen-specific immune responses and lead to successful tumor rejection in a prostate cancer mouse model (Willis *et al.*, 2001). These results encouraged us to further evaluate the effects of HSV amplicon transduction on DC biology as stated in this study, aiming to fine-tune this vaccine platform.

DCs have been the focus of vaccine development for years (Nouri-Shirazi *et al.*, 2000; Paczesny *et al.*, 2003; Moll, 2004; Palucka *et al.*, 2005). DC-based vaccines are more effective than peptide vaccines because of the nature of DCs in boosting immune responses as potent adjuvant (Mayordomo *et al.*, 1997). Combining HSV amplicons with DCs represents a strategy that could be more advantageous than conventional DC-based vaccination in many ways. First, HSV amplicons provide a rapid, facile, and efficient way of introducing antigens of interest into DCs rather than by use of peptide-pulsing protocols. This approach can overcome the main restriction in peptide-pulsed DC-based vaccines, in which the MHC haplotype is critical. Second, unlike other platforms such as those

FIG. 5. Dynamic changes in DC surface markers occur after HSV amplicon transduction. Day 9 BM-derived cells were harvested and subjected to HSVPrPuc amplicon transduction at an MOI of 2. Excessive amplicon particles were removed by thorough washing and amplicon-transduced cells were resuspended in medium in the presence of rGM-CSF. Aliquots of these cells were collected at 2-hr intervals after transduction and their phenotypes were analyzed by flow cytometric analysis. Quadrant analysis was gated on live cells (PI negative). Expression of CD11c and MHCII on BM-derived cells was monitored in untransduced control cultures (A, G, and M) and 0 hr (B, H, and N), 2 hr (C, I, and O), 4 hr (D, J, and P), 6 hr (E, K, and Q), and 8 hr (F, L, and R) after HSV amplicon transduction. Numbers shown in quadrants (A–F) represent the percentage of R3 (CD11c⁺MHCII⁺) in the gated live cell pool. Mean fluorescence intensity (MFI) profiles were also analyzed for MHCII⁺ cells (G–L) and CD11c⁺ cells (M–R). (S) Dynamic changes in MHC I, MHCII, CD11c, and CD80 markers on the BM-derived CD11c⁺MHCII⁺ cell subset (R3) were measured 0–8 hr after HSV amplicon transduction. The expression levels of each marker at various time points were first assessed by histogram analysis and compared with those profiles found on non-transduced cells. All flow cytometric analyses were performed independently three times and representative plots from a single experimental run are shown.

based on vaccinia virus-derived vaccines, whose transduction severely affects the surface expression of MHCII (Li *et al.*, 2005), transduction with HSV amplicons does not dampen the normal expression of MHC I and MHCII molecules on the surface of DCs (Willis *et al.*, 2001). Third, large antigens as well as immunomodulatory molecules can be delivered to DCs concurrently by a single HSV amplicon, because of its inherently high transgene capacity, providing higher flexibility in regulating immune responses (Bowers *et al.*, 2003).

Herpes simplex virus (HSV) gains its entry into cells by fusion of viral envelope with the cell surface membrane though a cascade of interactions involving multiple viral glycoproteins and cellular receptors (Bender *et al.*, 2005; Kwon

et al., 2006; Spear *et al.*, 2006). It has been demonstrated that DCs interact with HSV in a glycoprotein-dependent and Toll-like receptor-2 (TLR2)-independent manner (Reske *et al.*, 2008). On the basis of our result that the glycosylated form of HveC (nectin-1) was expressed on the surface of mouse cells (Lim *et al.*, 2008), we hypothesized that HveC might be the major receptor of DCs interacting with viral protein, and thus determined to examine the expression of HveC on BM-derived cells. In this study, we showed that the expression of HveC is highly restricted to the CD11c⁺MHCII⁺ DC population (Fig. 1C) among four distinct BM-derived cell populations (Fig. 1B). However, all these four subsets of BM-derived cells could be efficiently transduced with HSV amplicon

particles (Fig. 3), regardless of their surface expression of CD11c, MHCII, and HveC molecules. Although our results did not exclude the possibility of HveC serving as the cellular receptor of HSV entity, taken together they suggested that HveC might not be the major one. Blocking of surface HveC by antibody followed by examination of the transduction efficiency of HSV amplicon might provide us with direct clues regarding the role of HveC in HSV entry. Our data, however, suggest that another class of surface moieties, such as heparin sulfate moieties (Shukla *et al.*, 1999), polysaccharides known to facilitate HSV particle–target cell surface interactions, may be involved in mediating HSV amplicon entry into BM-derived DCs. Copeland and colleagues (2008) showed that O-sulfated heparin octasaccharide was able to inhibit the entry of HSV-1. It will be interesting to test whether this oligosaccharide can also block HSV amplicon transduction of BM-derived cells. In addition, in this study, we found that the CD11c⁺MHCII⁺ subset (R2; Fig. 3A) was transduced with HSV amplicon at the highest efficiency. This observation suggests that this cell subset might express unique cellular receptors or a polysaccharide moiety that promotes HSV entry. Further study of surface molecules expressed on this particular BM-derived cell subset might help us to identify novel proteins on cells involved in HSV transduction.

mDCs and iDCs differ in their ability to capture, process, and present antigen, as well as to activate T cells (Hartgers *et al.*, 2000; Jensen, 2005). This led us to examine whether mDCs and iDCs respond differently to HSV amplicon transduction, aiming to choose the most optimal physical conditions for DCs to develop the DC-HSV amplicon vaccine platform. Our results showed that mDCs and iDCs do respond differently to HSV transduction. For mDCs, the percentage of DC subset R3 (CD11c⁺MHCII⁺) was not changed by HSV transduction (Fig. 4). In contrast, the R3 subset percentage in iDCs was reduced sharply by HSV transduction. Once matured with LPS, mDCs resist HSV amplicon transduction-mediated change in their respective surface phenotypic profiles. It is likely that the maturation signal delivered by LPS desensitized mDCs in their response to additional maturation stimuli such as viral transduction. In addition, we found that the expression of MHCII was more susceptible than that of CD11c to HSV amplicon transduction on iDCs (Table 1). From the constant sum of R3 and R5 (Table 1 and Fig. 4C, right), we assumed that the majority of CD11c⁺MHCII⁺ (R3) cells began to lose their surface expression of MHCII and became positive only for CD11c (R5) after amplicon transduction. This observation is in line with the result that glycoprotein B (gB) on the viral envelope restrained the surface expression of MHCII by associating with the HLA-DR subunit (Neumann *et al.*, 2003). gB prevented cell surface expression of MHCII and caused significant changes in MHCII intracellular distribution (Neumann *et al.*, 2003). A similar scenario could be envisioned for the effect observed in iDCs transduced with amplicon virions if virion envelope-localized gB mediates this phenomenon, as differentiation between the effects of gB harbored in the envelope of incoming virions and gB expressed *de novo* during infection was not made by this prior study.

We observed a higher percentage of dead cells in the mDC pool even before HSV amplicon transduction (Fig. 4C). This could be explained by higher caspase-3 activity in mDCs, making them prone to apoptosis (Santambrogio *et al.*, 2005).

For both iDCs and mDCs, there was a sharp decrease in cell viability when viral MOI was elevated from 1 to 5 (Fig. 4C). These data indicate that an MOI higher than 5 should be avoided when establishing transduction conditions. However, on the other hand, MOIs lower than 0.5 should also be avoided, because the frequency at which transduced DCs will express the gene of interest delivered by HSV amplicon vector will be extremely low when viral particle number is limited. On the basis of our results concerning cell toxicity (Fig. 4C) and transduction efficiency (Fig. 2), we believe that the optimal MOI for DC transduction should lie between 2 and 4. Within this range, increasing cell lethality would ensure that transduction of DCs by HSV amplicon is taking place. At the same time, increased cell debris from apoptotic cells in this MOI range could be an advantage of this HSV amplicon, DC-based platform. It is possible that cell debris from these apoptotic cells expressing the antigen of interest could be internalized by other DCs, which will magnify the degree of antigen-specific immune response in a type of cross-presentation. Similar phenomena have been reported when DCs were pulsed with tumor debris instead of pure tumor antigens (Yamanaka *et al.*, 2001; Tamir *et al.*, 2007).

Besides HSV amplicon, transduction of DCs with lentiviral vectors (Koya *et al.*, 2003) and vaccinia virus (Yao *et al.*, 2007) also induces DC maturation. It is noteworthy that our current study demonstrates that HSV amplicon transduction promotes a transient “maturation-like” process in DCs (Fig. 5), whereas infection with wild-type HSV-1 inhibits DC maturation (Salio *et al.*, 1999). Although it is not yet entirely clear why DCs respond to HSV amplicon and HSV-1 in such disparate ways, we believe that the lack of encoded viral genes in the HSV amplicon genome may preclude the methods that wild-type HSV typically use to evade the immune system by downregulating MHC expression, blocking antigen processing, as well as hindering DC maturation (Salio *et al.*, 1999). When trying to establish a vaccine platform involving the transduction of DCs with HSV amplicon, it is necessary to take every component (expressed foreign or viral genes in vector, proteins obtained during viral packaging, etc.) present in the viral stock into consideration. Minor changes in either HSV amplicon preparation or transduction protocol will greatly affect the efficiency of DC transduction and thus the outcome of immune responses. This is supported by results published by our collaborators Santos and colleagues, showing that transduction efficiencies of HSV amplicon stocks could be modulated by the particular strain of origin for helper virus used for HSV amplicon packaging (Santos *et al.*, 2007).

In this study, we demonstrated that HSV amplicon transduction serves as a positive signal for limited DC maturation, as several DC surface markers (MHCI, MHCII, and CD11c) were transiently upregulated after transduction (Fig. 5S). Although there was a brief reduction in the surface expression of MHCI, MHCII, and CD11c shortly after transduction, cells resumed expression of these molecules at an enhanced level by 2 hr (Fig. 5S). Consistent with our observations, upregulation of several DC surface markers, such as CD40, CD83, MHCI, and MHCII, was previously reported after DCs were transduced with HSV-1 (Reske *et al.*, 2008) or adenoviral (Ad) vectors (Tan *et al.*, 2005). These phenotypical changes in iDCs by HSV amplicon transduction might reflect the functional alteration of DCs as previously suggested by Tan and

colleagues (2005). Although we did not further characterize the functional changes in HSV amplicon-transduced DCs in this study, it was shown previously that upregulation of costimulatory molecules such as CD80 and CD86 as well as MHC proteins on iDCs after adenoviral or lentiviral vector transduction correlated with an increased ability of DCs to act as stimulators in a mixed lymphocyte reaction (Tan *et al.*, 2005). On the basis of this report, we posit that a similar correlation between surface marker expression and immunoregulatory function might also apply to the response of iDCs to HSV amplicon transduction.

Interestingly, although our data showed that HSV amplicon transduction induced transient upregulation of both MHCII and MHCI expression, the co-upregulation of MHCII and MHCI might not comport with the response of DCs transduced with other viral platforms. Yao and colleagues (2007) showed that vaccinia viral (VV) infection causes an upregulation of MHCI but a downregulation of MHCII on DCs in live animals (Yao *et al.*, 2007). Together, these data suggested that VV and amplicon might employ different mechanisms in modulating the antigen presentation activity of iDCs as represented by their variations in regulating surface MHCII expression. On the other hand, however, it is also likely that the discrepancy between the effects of VV and HSV amplicon transduction on MHCII expression may simply be due to the difference in experimental setup (live animals vs. *ex vivo*-cultured cells).

Our results demonstrated that the function of HSV amplicon-transduced DCs declined dramatically 8 hr posttransduction, as represented by a sharp decrease in the surface expression of CD11c and several other DC surface markers (Fig. 5R and S). On the basis of this result, we believe that HSV amplicon-transduced DCs are no longer effective 8 hr after transduction for boosting immune responses in animals. The optimal time of administering HSV amplicon-transduced DCs into animals to achieve the maximal vaccination efficacy should be between 2 and 4 hr posttransduction.

In conclusion, our data demonstrate that HSV amplicons were able to transduce DCs efficiently, that iDCs and mDCs respond differently to HSV amplicon transduction, and that HSV amplicon transduction induced transient phenotypic changes in DCs that portend maturation. Results from our current study will facilitate fine-tuning of the DC-HSV amplicon vaccination platform and exploit its potential in future immunotherapy.

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Author Disclosure Statement

No competing financial interests exist.

References

- Alnaeeli, M., Park, J., Mahamed, D., Penninger, J.M., and Teng, Y.T. (2007). Dendritic cells at the osteo-immune interface: Implications for inflammation-induced bone loss. *J. Bone Miner. Res.* 22, 775–780.
- Bender, F.C., Whitbeck, J.C., Lou, H., Cohen, G.H., and Eisenberg, R.J. (2005). Herpes simplex virus glycoprotein B binds to cell surfaces independently of heparan sulfate and blocks virus entry. *J. Virol.* 79, 11588–11597.
- Bennett, J.J., Delman, K.A., Burt, B.M., Mariotti, A., Malhotra, S., Zager, J., Petrowsky, H., Mastorides, S., Federoff, H., and Fong, Y. (2002). Comparison of safety, delivery, and efficacy of two oncolytic herpes viruses (G207 and NV1020) for peritoneal cancer. *Cancer Gene Ther.* 9, 935–945.
- Bowers, W.J., Howard, D.F., and Federoff, H.J. (2000). Discordance between expression and genome transfer titering of HSV amplicon vectors: Recommendation for standardized enumeration. *Mol. Ther.* 1, 294–299.
- Bowers, W.J., Howard, D.F., Brooks, A.I., Halterman, M.W., and Federoff, H.J. (2001). Expression of vhs and VP16 during HSV-1 helper virus-free amplicon packaging enhances titers. *Gene Ther.* 8, 111–120.
- Bowers, W.J., Chen, X., Guo, H., Frisina, D.R., Federoff, H.J., and Frisina, R.D. (2002). Neurotrophin-3 transduction attenuates cisplatin spiral ganglion neuron ototoxicity in the cochlea. *Mol. Ther.* 6, 12–18.
- Bowers, W.J., Olschowka, J.A., and Federoff, H.J. (2003). Immune responses to replication-defective HSV-1 type vectors within the CNS: Implications for gene therapy. *Gene Ther.* 10, 941–945.
- Bowers, W.J., Mastrangelo, M.A., Stanley, H.A., Casey, A.E., Milo, L.J., Jr., and Federoff, H.J. (2005). HSV amplicon-mediated A β vaccination in Tg2576 mice: Differential antigen-specific immune responses. *Neurobiol. Aging* 26, 393–407.
- Buchsel, P.C., and DeMeyer, E.S. (2006). Dendritic cells: Emerging roles in tumor immunotherapy. *Clin. J. Oncol. Nurs.* 10, 629–640.
- Carew, J.F., Kooby, D.A., Halterman, M.W., Kim, S.H., Federoff, H.J., and Fong, Y. (2001). A novel approach to cancer therapy using an oncolytic herpes virus to package amplicons containing cytokine genes. *Mol. Ther.* 4, 250–256.
- Caux, C., Ait-Yahia, S., Chemin, K., De Bouteiller, O., Dieu-Nosjean, M.C., Homey, B., Massacrier, C., Vanbervliet, B., Zlotnik, A., and Vicari, A. (2000). Dendritic cell biology and regulation of dendritic cell trafficking by chemokines. *Springer Semin. Immunopathol.* 22, 345–369.
- Celluzzi, C.M., and Faló, L.D., Jr. (1998). Physical interaction between dendritic cells and tumor cells results in an immunogen that induces protective and therapeutic tumor rejection. *J. Immunol.* 160, 3081–3085.
- Celluzzi, C.M., Mayordomo, J.I., Storkus, W.J., Lotze, M.T., and Faló, L.D., Jr. (1996). Peptide-pulsed dendritic cells induce antigen-specific CTL-mediated protective tumor immunity. *J. Exp. Med.* 183, 283–287.
- Condon, C., Watkins, S.C., Celluzzi, C.M., Thompson, K., and Faló, L.D., Jr. (1996). DNA-based immunization by *in vivo* transfection of dendritic cells. *Nat. Med.* 2, 1122–1128.
- Copeland, R., Balasubramaniam, A., Tiwari, V., Zhang, F., Bridges, A., Linhardt, R.J., Shukla, D., and Liu, J. (2008). Using a 3-O-sulfated heparin octasaccharide to inhibit the entry of herpes simplex virus type 1. *Biochemistry* 47, 5774–5783.
- de Jong, E.C., Smits, H.H., and Kapsenberg, M.L. (2005). Dendritic cell-mediated T cell polarization. *Springer Semin. Immunopathol.* 26, 289–307.
- Delman, K.A., Zager, J.S., Bennett, J.J., Malhotra, S., Ebricht, M.I., McAuliffe, P.F., Halterman, M.W., Federoff, H.J., and Fong, Y.

- (2002). Efficacy of multiagent herpes simplex virus amplicon-mediated immunotherapy as adjuvant treatment for experimental hepatic cancer. *Ann. Surg.* 236, 337–342; discussion 342–333.
- Detrait, E.R., Bowers, W.J., Halterman, M.W., Giuliano, R.E., Bennice, L., Federoff, H.J., and Richfield, E.K. (2002). Reporter gene transfer induces apoptosis in primary cortical neurons. *Mol. Ther.* 5, 723–730.
- Di Nicola, M., Carlo-Stella, C., Anichini, A., Mortarini, R., Guidetti, A., Tragni, G., Gallino, F., Del Vecchio, M., Ravnagnani, F., Morelli, D., Chaplin, P., Arndtz, N., Sutter, G., Drexler, I., Parmiani, G., Cascinelli, N., and Gianni, A.M. (2003). Immunization of patients with malignant melanoma with autologous CD34⁺ cell-derived dendritic cells transduced *ex vivo* with a recombinant replication-deficient vaccinia vector encoding the human tyrosinase gene: A phase I trial [clinical protocol]. *Hum. Gene Ther.* 14, 1347–1360.
- Dubsky, P., Ueno, H., Piqueras, B., Connolly, J., Banchereau, J., and Palucka, A.K. (2005). Human dendritic cell subsets for vaccination. *J. Clin. Immunol.* 25, 551–572.
- Fajardo-Moser, M., Berzel, S., and Moll, H. (2008). Mechanisms of dendritic cell-based vaccination against infection. *Int. J. Med. Microbiol.* 298, 11–20.
- Figdor, C.G., De Vries, I.J., Lesterhuis, W.J., and Melief, C.J. (2004). Dendritic cell immunotherapy: Mapping the way. *Nat. Med.* 10, 475–480.
- Galli, S.J., Tsai, M., and Piliponsky, A.M. (2008). The development of allergic inflammation. *Nature* 454, 445–454.
- Geschwind, M.D., Hartnick, C.J., Liu, W., Amat, J., Van De Water, T.R., and Federoff, H.J. (1996). Defective HSV-1 vector expressing BDNF in auditory ganglia elicits neurite outgrowth: Model for treatment of neuron loss following cochlear degeneration. *Hum. Gene Ther.* 7, 173–182.
- Gordon, S., and Taylor, P.R. (2005). Monocyte and macrophage heterogeneity. *Nat. Rev. Immunol.* 5, 953–964.
- Guermonprez, P., Valladeau, J., Zitvogel, L., Thery, C., and Amigorena, S. (2002). Antigen presentation and T cell stimulation by dendritic cells. *Annu. Rev. Immunol.* 20, 621–667.
- Hartgers, F.C., Figdor, C.G., and Adema, G.J. (2000). Towards a molecular understanding of dendritic cell immunobiology. *Immunol. Today* 21, 542–545.
- He, Y., Zhang, J., Mi, Z., Robbins, P., and Falo, L.D., Jr. (2005). Immunization with lentiviral vector-transduced dendritic cells induces strong and long-lasting T cell responses and therapeutic immunity. *J. Immunol.* 174, 3808–3817.
- Inaba, K., Metlay, J.P., Crowley, M.T., and Steinman, R.M. (1990). Dendritic cells pulsed with protein antigens *in vitro* can prime antigen-specific, MHC-restricted T cells *in situ*. *J. Exp. Med.* 172, 631–640.
- Iwashita, Y., Goto, S., Tominaga, M., Sasaki, A., Ohmori, N., Goto, T., Sato, S., Ohta, M., and Kitano, S. (2005). Dendritic cell immunotherapy with poly(D,L-2,4-diaminobutyric acid)-mediated intratumoral delivery of the interleukin-12 gene suppresses tumor growth significantly. *Cancer Sci.* 96, 303–307.
- Jensen, P.E. (2005). Dendritic cells: Caspases keep them young. *Nat. Immunol.* 6, 968–969.
- Johnson, D.C., Ghosh-Choudhury, G., Smiley, J.R., Fallis, L., and Graham, F.L. (1988). Abundant expression of herpes simplex virus glycoprotein gB using an adenovirus vector. *Virology* 164, 1–14.
- Koya, R.C., Kasahara, N., Favaro, P.M., Lau, R., Ta, H.Q., Weber, J.S., and Stripecke, R. (2003). Potent maturation of monocyte-derived dendritic cells after CD40L lentiviral gene delivery. *J. Immunother.* 26, 451–460.
- Koya, R.C., Weber, J.S., Kasahara, N., Lau, R., Villacres, M.C., Levine, A.M., and Stripecke, R. (2004). Making dendritic cells from the inside out: Lentiviral vector-mediated gene delivery of granulocyte-macrophage colony-stimulating factor and interleukin 4 into CD14⁺ monocytes generates dendritic cells *in vitro*. *Hum. Gene Ther.* 15, 733–748.
- Krummenacher, C., Baribaud, I., Ponce De Leon, M., Whitbeck, J.C., Lou, H., Cohen, G.H., and Eisenberg, R.J. (2000). Localization of a binding site for herpes simplex virus glycoprotein D on herpesvirus entry mediator C by using antireceptor monoclonal antibodies. *J. Virol.* 74, 10863–10872.
- Kwon, H., Bai, Q., Baek, H.J., Felmet, K., Burton, E.A., Goins, W.F., Cohen, J.B., and Glorioso, J.C. (2006). Soluble V domain of Nectin-1/HveC enables entry of herpes simplex virus type 1 (HSV-1) into HSV-resistant cells by binding to viral glycoprotein D. *J. Virol.* 80, 138–148.
- Li, P., Wang, N., Zhou, D., Yee, C.S., Chang, C.H., Brutkiewicz, R.R., and Blum, J.S. (2005). Disruption of MHC class II-restricted antigen presentation by vaccinia virus. *J. Immunol.* 175, 6481–6488.
- Lim, S.T., Lim, K.C., Giuliano, R.E., and Federoff, H.J. (2008). Temporal and spatial localization of nectin-1 and I-fafadin during synaptogenesis in hippocampal neurons. *J. Comp. Neurol.* 507, 1228–1244.
- Liu, Y., Chiriva-Internati, M., Grizzi, F., Salati, E., Roman, J.J., Lim, S., and Hermonat, P.L. (2001). Rapid induction of cytotoxic T-cell response against cervical cancer cells by human papillomavirus type 16 E6 antigen gene delivery into human dendritic cells by an adeno-associated virus vector. *Cancer Gene Ther.* 8, 948–957.
- Lutz, M.B., Kukutsch, N., Ogilvie, A.L., Rossner, S., Koch, F., Romani, N., and Schuler, G. (1999). An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J. Immunol. Methods* 223, 77–92.
- Mahnke, K., and Enk, A.H. (2005). Dendritic cells: Key cells for the induction of regulatory T cells? *Curr. Top. Microbiol. Immunol.* 293, 133–150.
- Mayordomo, J.I., Zorina, T., Storkus, W.J., Zitvogel, L., Celluzzi, C., Falo, L.D., Melief, C.J., Ildstad, S.T., Kast, W.M., Deleo, A.B., and Lotze, M.T. (1995). Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic antitumour immunity. *Nat. Med.* 1, 1297–1302.
- Mayordomo, J.I., Zorina, T., Storkus, W.J., Zitvogel, L., Garcia-Prats, M.D., Deleo, A.B., and Lotze, M.T. (1997). Bone marrow-derived dendritic cells serve as potent adjuvants for peptide-based antitumor vaccines. *Stem Cells* 15, 94–103.
- Moll, H. (2004). Antigen delivery by dendritic cells. *Int. J. Med. Microbiol.* 294, 337–344.
- Moser, M., and Murphy, K.M. (2000). Dendritic cell regulation of T_H1–T_H2 development. *Nat. Immunol.* 1, 199–205.
- Neumann, J., Eis-Hubinger, A.M., and Koch, N. (2003). Herpes simplex virus type 1 targets the MHC class II processing pathway for immune evasion. *J. Immunol.* 171, 3075–3083.
- Nouri-Shirazi, M., Banchereau, J., Fay, J., and Palucka, K. (2000). Dendritic cell based tumor vaccines. *Immunol. Lett.* 74, 5–10.
- Oehmig, A., Fraefel, C., and Breakefield, X.O. (2004a). Update on herpesvirus amplicon vectors. *Mol. Ther.* 10, 630–643.
- Oehmig, A., Fraefel, C., Breakefield, X.O., and Ackermann, M. (2004b). Herpes simplex virus type 1 amplicons and their hybrid virus partners, EBV, AAV, and retrovirus. *Curr. Gene Ther.* 4, 385–408.

- Paczesny, S., Ueno, H., Fay, J., Banchereau, J., and Palucka, A.K. (2003). Dendritic cells as vectors for immunotherapy of cancer. *Semin. Cancer Biol.* 13, 439–447.
- Palucka, A.K., Laupeze, B., Asford, C., Saito, H., Jego, G., Fay, J., Paczesny, S., Pascual, V., and Banchereau, J. (2005). Immunotherapy via dendritic cells. *Adv. Exp. Med. Biol.* 560, 105–114.
- Reske, A., Pollara, G., Krummenacher, C., Katz, D.R., and Chain, B.M. (2008). Glycoprotein-dependent and TLR2-independent innate immune recognition of herpes simplex virus-1 by dendritic cells. *J. Immunol.* 180, 7525–7536.
- Salio, M., Cella, M., Suter, M., and Lanzavecchia, A. (1999). Inhibition of dendritic cell maturation by herpes simplex virus. *Eur. J. Immunol.* 29, 3245–3253.
- Santambrogio, L., Potolicchio, I., Fessler, S.P., Wong, S.H., Raposo, G., and Strominger, J.L. (2005). Involvement of caspase-cleaved and intact adaptor protein 1 complex in endosomal remodeling in maturing dendritic cells. *Nat. Immunol.* 6, 1020–1028.
- Santos, K., Sanfilippo, C.M., Narrow, W.C., Casey, A.E., Rodriguez-Colon, S.M., McDermott, M.P., Federoff, H.J., Bowers, W.J., and Dewhurst, S. (2007). Infectivity of herpes simplex virus type-1 (HSV-1) amplicon vectors in dendritic cells is determined by the helper virus strain used for packaging. *J. Virol. Methods* 145, 37–46.
- Shukla, D., Liu, J., Blaiklock, P., Shworak, N.W., Bai, X., Esko, J.D., Cohen, G.H., Eisenberg, R.J., Rosenberg, R.D., and Spear, P.G. (1999). A novel role for 3-O-sulfated heparan sulfate in herpes simplex virus 1 entry. *Cell* 99, 13–22.
- Sortwell, C.E., Bowers, W.J., Counts, S.E., Pitzer, M.R., Fleming, M.F., McGuire, S.O., Maguire-Zeiss, K.A., Federoff, H.J., and Collier, T.J. (2007). Effects of *ex vivo* transduction of mesencephalic reagggregates with bcl-2 on grafted dopamine neuron survival. *Brain Res.* 1134, 33–44.
- Spear, P.G., Manoj, S., Yoon, M., Jogger, C.R., Zago, A., and Myscowski, D. (2006). Different receptors binding to distinct interfaces on herpes simplex virus gD can trigger events leading to cell fusion and viral entry. *Virology* 344, 17–24.
- Takayama, T., Tahara, H., and Thomson, A.W. (1999). Transduction of dendritic cell progenitors with a retroviral vector encoding viral interleukin-10 and enhanced green fluorescent protein allows purification of potentially tolerogenic antigen-presenting cells. *Transplantation* 68, 1903–1909.
- Tamir, A., Basagila, E., Kagahzian, A., Jiao, L., Jensen, S., Nicholls, J., Tate, P., Stamp, G., Farzaneh, F., Harrison, P., Stauss, H., George, A.J., Habib, N., Lechler, R.I., and Lombardi, G. (2007). Induction of tumor-specific T-cell responses by vaccination with tumor lysate-loaded dendritic cells in colorectal cancer patients with carcinoembryonic-antigen positive tumors. *Cancer Immunol. Immunother.* 56, 2003–2016.
- Tan, H., Zeng, L.J., and Ye, X. (2005). *Ex vivo* induction of anti-leukemia cytotoxic T cell effect by dendritic cells from human umbilical cord blood cell origin. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 13, 472–478.
- Taylor, P.R., and Gordon, S. (2003). Monocyte heterogeneity and innate immunity. *Immunity* 19, 2–4.
- Terry-Allison, T., Montgomery, R.I., Whitbeck, J.C., Xu, R., Cohen, G.H., Eisenberg, R.J., and Spear, P.G. (1998). HveA (herpesvirus entry mediator A), a coreceptor for herpes simplex virus entry, also participates in virus-induced cell fusion. *J. Virol.* 72, 5802–5810.
- Timares, L., Douglas, J.T., Tillman, B.W., Krasnykh, V., and Curiel, D.T. (2004). Adenovirus-mediated gene delivery to dendritic cells. *Methods Mol. Biol.* 246, 139–154.
- Wade-Martins, R., Frampton, J., and James, M.R. (1999). Long-term stability of large insert genomic DNA episomal shuttle vectors in human cells. *Nucleic Acids Res.* 27, 1674–1682.
- Wade-Martins, R., Smith, E.R., Tyminski, E., Chiocca, E.A., and Saeki, Y. (2001). An infectious transfer and expression system for genomic DNA loci in human and mouse cells. *Nat. Biotechnol.* 19, 1067–1070.
- Wade-Martins, R., Saeki, Y., and Chiocca, E.A. (2003). Infectious delivery of a 135-kb LDLR genomic locus leads to regulated complementation of low-density lipoprotein receptor deficiency in human cells. *Mol. Ther.* 7, 604–612.
- Willis, R.A., Bowers, W.J., Turner, M.J., Fisher, T.L., Abdul-Alim, C.S., Howard, D.F., Federoff, H.J., Lord, E.M., and Frelinger, J.G. (2001). Dendritic cells transduced with HSV-1 amplicons expressing prostate-specific antigen generate antitumor immunity in mice. *Hum. Gene Ther.* 12, 1867–1879.
- Yamanaka, R., Zullo, S.A., Tanaka, R., Blaese, M., and Xanthopoulos, K.G. (2001). Enhancement of antitumor immune response in glioma models in mice by genetically modified dendritic cells pulsed with Semliki Forest virus-mediated complementary DNA. *J. Neurosurg.* 94, 474–481.
- Yao, Y., Li, P., Singh, P., Thiele, A.T., Wilkes, D.S., Renukaradhya, G.J., Brutkiewicz, R.R., Travers, J.B., Luker, G.D., Hong, S.C., Blum, J.S., and Chang, C.H. (2007). Vaccinia virus infection induces dendritic cell maturation but inhibits antigen presentation by MHC class II. *Cell. Immunol.* 246, 92–102.
- Zager, J.S., Delman, K.A., Malhotra, S., Ebright, M.I., Bennett, J.J., Kates, T., Halterman, M., Federoff, H., and Fong, Y. (2001). Combination vascular delivery of herpes simplex oncolytic viruses and amplicon mediated cytokine gene transfer is effective therapy for experimental liver cancer. *Mol. Med.* 7, 561–568.
- Zhang, W., Yang, H., Wang, Z., and Jim, X. (2002). TNF- α gene-modified dendritic cells act as more potent adjuvants for peptide delivery to induce specific antitumor immunity in mice. *Chin. Med. J. (Engl)* 115, 1767–1771.
- Ziegler-Heitbrock, H.W. (2000). Definition of human blood monocytes. *J. Leukoc. Biol.* 67, 603–606.

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