

Measles Virus Suppresses Cell-mediated Immunity by Interfering with the Survival and Functions of Dendritic and T Cells

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Summary

Secondary infections due to a marked immunosuppression have long been recognized as a major cause of the high morbidity and mortality rate associated with acute measles. The mechanisms underlying the inhibition of cell-mediated immunity are not clearly understood but dysfunctions of monocytes as antigen-presenting cells (APC) are implicated. In this report, we demonstrate that measles virus (MV) replicates weakly in the resting dendritic cells (DC) as in lipopolysaccharide-activated monocytes, but intensively in CD40-activated DC. The interaction of MV-infected DC with T cells not only induces syncytia formation where MV undergoes massive replication, but also leads to an impairment of DC and T cell function and cell death. CD40-activated DC decrease their capacity to produce interleukin (IL) 12, and T cells are unable to proliferate in response to MV-infected DC stimulation. A massive apoptosis of both DC and T cells is observed in the MV pulsed DC-T cell cocultures. This study suggests that DC represent a major target of MV. The enhanced MV replication during DC-T cell interaction, leading to an IL-12 production decrease and the deletion of DC and T cells, may be the essential mechanism of immunosuppression induced by MV.

In developing countries, measles virus (MV)¹ infection remains a significant cause of mortality accounting for more than one million deaths per year, especially among young children. Although malnutrition and an insufficient supply of medicines may contribute to the severity of measles in the young, it is mainly due to secondary infections (1–3). Increased susceptibility to secondary infection correlates with depressed cell-mediated immunity after measles. These immunologic abnormalities were first described by von Pirquet in 1908 when he noticed a decrease in tuberculin skin reactivity in measles patients (4). The mechanism of immune suppression is poorly understood but it is widely assumed that immune suppression is mainly due to MV replication in leukocytes (5). Infected T cells and monocytes die by apoptosis (6–8) particularly within syncytia (8). Syncytia formation is due to the cytopathic effect of MV (9, 10). The T and B lymphocytes from the PBL of measles

patients present a reduced proliferation capacity in response to polyclonal stimulation (11, 12) due to a cell cycle arrest in the end of the G1 phase. In measles, T cells are skewed from type 1 responses (cell-mediated immunity) towards type 2 responses (antibody-mediated immunity) (13, 14). There is *in vivo* and *in vitro* evidence of a Th2 polarization in cytokine responses during and after measles: production of IL-4 increases while productions of IL-2 and IFN- γ decrease (14).

This Th2 polarization is suggested to be partly due to the decreased ability of monocytes to produce TNF- α and IL-12 (15, 16). Earlier studies have suggested that the immunodeficiency associated with MV infection could be related in part to the dysfunction of APCs such as monocytes or B cells (11, 17, 18). Nevertheless, the role played by dendritic cells (DC), which are the sole professional APCs able to prime naive T lymphocytes (19–21), has not yet been studied in measles.

DC are professional APCs, which capture and process antigens at their immature stage and present antigen to naive T cells to initiate T cell-dependent immune responses upon their maturation (22, 23). The high capacity of mature DC to stimulate naive T cells has been attributed to a variety

¹Abbreviations used in this paper: DC, dendritic cells; L, ligand; L⁺, ligand-transfected; MV, measles virus; NP, nucleoprotein; UV-MV, ultraviolet-inactivated measles virus.

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of factors like the expression of MHC class II molecules, CD80, CD86, CD40, and diverse adhesion molecules, which may favor TCR engagement and costimulation (24–27).

In this study, we used DC, generated by culturing monocytes in the presence of GM-CSF and IL-4 (28, 29), to address the following questions: (a) Does MV infect DC as effectively as monocytes?; (b) Do MV-infected DC transmit infection to syngenic T cells?; (c) What are the factors which modulate MV replication in the host cells?; and (d) How does MV infection interfere with the survival and function of DC and T cells?

Materials and Methods

Reagents. Anti-human CD40-ligand (mAb LL2, generated by Dr. Cees van Kooten at Schering-Plough Laboratory for Immunological Research, Dardilly, France) and anti-CD46 (mAb 20.6, reference 30) were used at 5 and 10 $\mu\text{g/ml}$ final concentrations, respectively. The control antibody mAb 30N (Schering-Plough Laboratory for Immunological Research) was used at 10 $\mu\text{g/ml}$.

Cell Purifications. Human peripheral blood was obtained from the Etablissement de Transfusion Sanguine (Lyon, France). Mononuclear cells were isolated by density gradient centrifugation using Ficoll/Hypaque, then centrifuged through a 50% Percoll gradient (Pharmacia Fine Chemicals, Uppsala, Sweden) for 20 min at 400 g . The light density fraction from the interface and the high density fraction from the pellet were recovered and incubated for 10 min at room temperature in 3% human serum-PBS. Monocytes were purified from the light density fraction by immunomagnetic depletion (Dyna, Oslo, Norway) using a cocktail of mAbs anti-CD19 (4G7 hybridoma, a gift from Dr. Ron Levy), CD3 (OKT3; American Type Culture Collection, Rockville, MD), and CD56 (NKH1, Coulter Corp., Miami, FL). The recovered monocytes were >91% pure as shown by flow cytometry with anti-CD14-FITC (Immunotech, Marseille, France). T lymphocytes were purified, from the high density fraction, by immunomagnetic depletion using a cocktail of mAbs anti-CD19, anti-CD40 (Schering-Plough Research Institute), and anti-CD56 (Coulter), anti-CD14, anti-CD16, anti-HLA-DR, and anti-glycophorin A (Immunotech). After two rounds of depletion, T lymphocytes were >98% pure as shown by flow cytometry with anti-CD3-FITC (Immunotech). As assessed by CD1a (Ortho Diagnostic Systems, Inc., Raritan, NJ) staining >95%, DC can be generated *in vitro* after 6 d of culture of 5×10^5 monocytes/ml plus 200 ng/ml GM-CSF and 50 U/ml IL-4 (Schering-Plough Research Institute) in 24-well flat-bottomed microtiter plates (Falcon Labware, Oxnard, CA).

Cell Cultures. All cultures were performed in 96-well flat-bottomed microtiter plates in a total volume of 200 μl and cultured in RPMI 1640 (Gibco Life Technologies, Inc., Grand Island, NY) supplemented with 10 mM Hepes (Gibco Life Technologies), 2 mM L-glutamine (Gibco Life Technologies), 40 $\mu\text{g/ml}$ gentamicin (Schering-Plough, Levallois-Perret, France), and 10% FCS (Gibco Life Technologies). T cells were activated with a combination of 10 ng/ml PMA (Sigma Chemical Co., St. Louis, MO) and 1 $\mu\text{g/ml}$ ionomycin (Sigma Chemical Co.) for 6 h and monocytes were activated with 1 $\mu\text{g/ml}$ LPS (*Escherichia coli* serotype O127:B8; Sigma Chemical Co.) overnight. After activation, cells were washed three times. Monocytes or DC alone were cultured at 2×10^5 cells/well. In the T cell cocultures, 10^5 monocytes or DC per well were cultured together with 10^5 T

cells/well. In the murine fibroblast cocultures, 2×10^5 monocytes or DC per well were cultured in the presence of 4×10^4 irradiated (7,000 rads) fibroblastic L cells or CD40 ligand-transfected L cells or CD28 ligand-transfected L cells (CD40L⁺ and CD28L⁺ cells; provided by Dr. Cees van Kooten, Schering-Plough Laboratory for Immunological Research and Dr. Lewis Lanier, DNAX Research Institute, Palo Alto, CA, respectively). 10 U/ml M-CSF (Genzyme Corp., Cambridge, MA) were added to the cultures containing monocytes. 200 ng/ml GM-CSF and 50 U/ml IL-4 were added to the cultures containing DC.

MV Infection and Detection. Monocytes and DC were infected with 0.1 PFU/cell of Vero cell-derived MV Hallé (Hallé strain is classified with the vaccine MV strain Edmonston, see reference 31), either with infectious virus or with neutralized virus by 254 nm ultraviolet rays for 30 min (UV-MV). After a 3-h incubation at 37°C, the cells were washed four times to be free of unattached virus. Then infected monocytes or DC were put in culture. Virus contents of culture supernatants were quantified by limiting dilution from 10 to 10 until 10^{-10} on confluent Vero cells. A single plaque in the Vero cells confluent culture represents one PFU generated by an individual infectious virus.

IL-12 Titration. Cell-free culture supernatants were harvested at various time points. IL-12p70 was measured by a specific ELISA kit (R&D Systems, Minneapolis, MN), limit of sensitivity 5 pg/ml. Tests were carried out in triplicate and standard deviations are indicated in the figure legends.

Proliferation Assay and Cell and Syncytia Counts. Cell numbers were determined with trypan blue dye and syncytia numbers were counted *in situ*. DNA synthesis was assessed at different time points as indicated, after an 8-h pulse with 1 μCi [³H]TdR. Results were expressed as the mean cpm \pm SD of triplicate cultures.

FACS[®] Immunostaining. After 15 min of permeabilization with 0.33% Saponin (Sigma Chemical Co.), cells were stained with anti-nucleoprotein (NP) viral protein provided by F. Wild (Institut Pasteur de Lyon, France; reference 32), followed by incubation with PE-labeled anti-mouse Ig (Immunotech). The ApoptTag[™] *in situ* apoptosis detection kit (S7110-KIT; Oncor Inc., Gaithersburg, MD) was used to detect apoptotic cells by fluorescence detection of digoxigenin-labeled genomic DNA. Anti-CD3, anti-CD14, and anti-DR-FITC-labeled mAbs used to verify the purity of T, monocytes, and DC, respectively, were from Immunotech.

Immunoenzymatic Staining. Cytospin preparations of 7×10^5 cells were fixed in acetone for 10 min at 4°C. The slides were washed in PBS. Double staining was done using mouse IgG1 anti-CD3, anti-CD13, or anti-HLA-DR (Immunotech) and mouse IgG2a anti-NP. Mouse IgG1 antibodies were revealed by sheep anti-mouse IgG1 (The Binding Site, Birmingham, UK) followed by mouse antialkaline phosphatase-alkaline phosphatase complexes (APAAP technique; Dako S.A., Trappes, France). NP staining was revealed by sheep anti-mouse IgG2a-biotin (The Binding Site) followed by ExtrAvidin peroxidase (Sigma Chemical Co.). Alkaline phosphatase activity was first developed by Fast blue substrate; peroxidase activity was developed with AEC (Sigma Chemical Co.) substrate. Two slides were incubated with mouse IgG1 and IgG2a isotype control mAbs (Dako S.A.). All of the slides were washed in tap water before mounting in glycerol (Dako S.A.).

Results

MV Replicates in DC and Induces Syncytia Formation. Both monocytes isolated from PBL and monocyte-derived

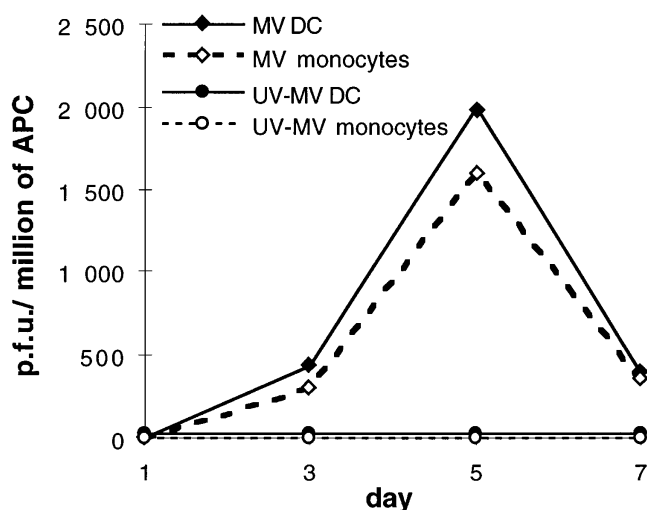


Figure 1. MV can replicate in DC as well as in monocytes. Kinetics of viral particle productions by 10^6 monocytes or by 10^6 DC pulsed with UV-MV or infected with MV. Infectious virus produced in the culture supernatants was quantified by the number of PFU on Vero cell lawn. Results are representative of six experiments. SD were $<10\%$ of variation.

DC have been infected by the MV at 0.1 PFU/cell. According to the kinetics of viral particle productions, measured by the number of PFU (Fig. 1), the level of MV replication was equivalent in both populations and reached a maximum level at day 5. By immunocytochemistry on cytopins, the detection of MV-NP (which is the earliest viral protein produced) in both populations confirmed that MV could replicate in monocytes as well as in DC (Fig. 2, A and B) *in vitro*. The wild-type strain of MV, replicated as well as vaccinal strain (Hallé) (data not shown). We noticed the presence of syncytia only on the cytopins of MV-infected DC (Fig. 2 B) but not in MV-infected monocytes. Syncytia formation in DC cultures depended on virus replication, since no syncytia were observed in DC pulsed with UV-MV (Fig. 2 A).

CD40-CD40 Ligand (CD40L) Interaction Induces a Burst of MV Production in DC-T Cell Cocultures. To analyze how MV replication was affected during interactions between monocytes and T cells or DC and T cells, monocytes or DC were infected with MV, then cocultured for up to 7 d with syngenic T cells activated with PMA plus ionomycin. PFU was measured at day 5 of the cocultures (the peak time point of viral production). In the presence of T cells, the viral particle productions were greatly increased in both cultures (Fig. 3 A). While viral production in monocytes increased fourfold, viral production in DC increased 18-fold in the T cell cocultures.

To identify the signal(s) from activated T cells promoting measles replication in these cocultures, we used an anti-CD40L antibody which blocks CD40-CD40L interactions. Infected monocytes or DC were cultured with activated T cells plus anti-CD40L or with an irrelevant antibody (Fig. 3 A). At day 5, anti-CD40L blocked 38 and 45% of PFU productions within monocyte-T cell and DC-T cell cocultures, respectively. Although increased doses of anti-

CD40L antibody failed to completely inhibit the T cell-dependent increases of viral production in these cocultures, CD40L⁺ L cells could replace activated T cells in promoting PFU production in DC or monocyte cocultures (Fig. 3, B and C). This suggested that the anti-CD40L antibody used might recognize one functional epitope of CD40L. Alternatively, other T cell-derived signals might be involved. However, CD28⁺ L cells were not shown to promote MV production in DC or monocytes (data not shown).

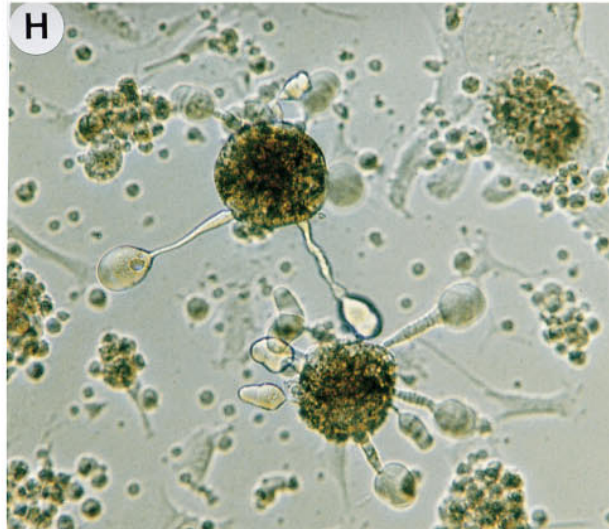
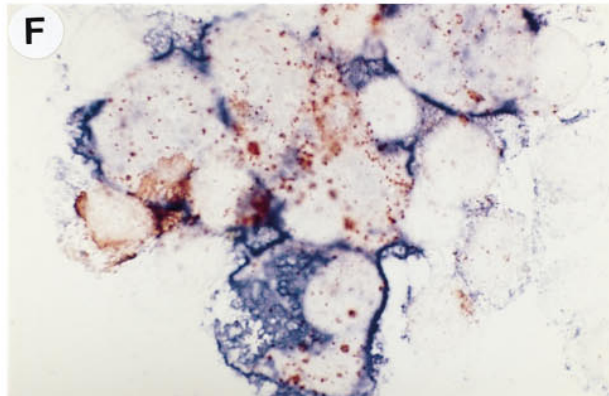
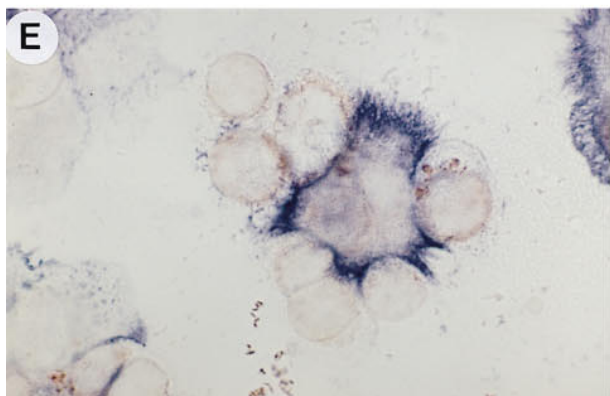
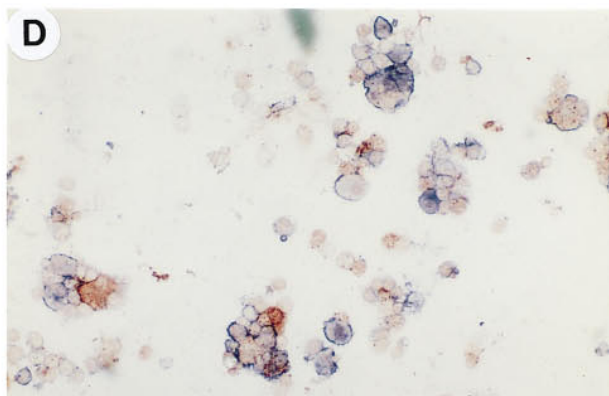
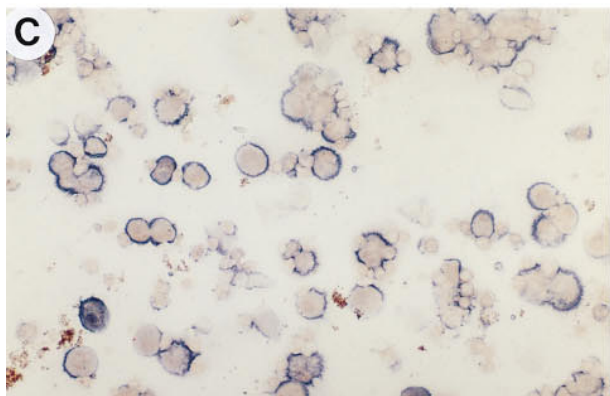
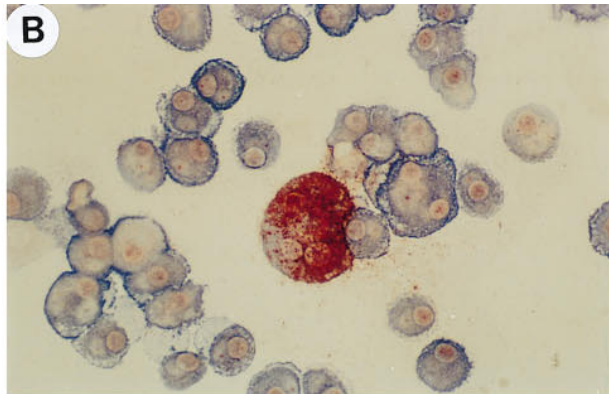
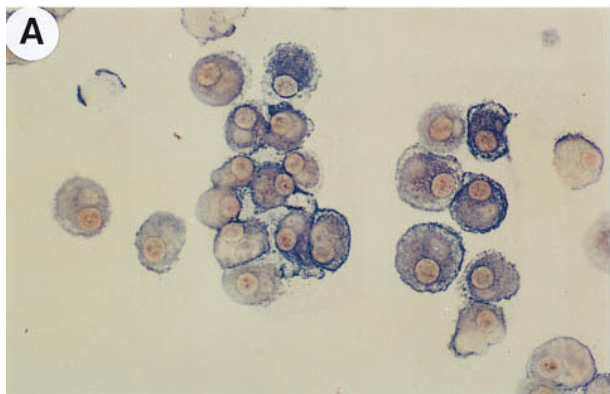
The presence of activated T cells or CD40L⁺ L cells increased both the size and the number of multinuclear syncytia in MV-infected DC cocultures (Fig. 2, G and H). The number of syncytia in DC-T cell or DC-CD40L⁺ L cell cocultures was 7-15-fold more than in DC cultured alone. The formation of syncytia in DC-T cell cocultures was partially inhibited by the anti-CD40L antibody (32-43% of inhibition, data not shown). All syncytia contained viral particles as demonstrated by immunohistological staining of NP antigen (Fig. 2, B, D, and F). The infected DC displayed poor DC morphology and viability (compare Fig. 2, D and F with C and E).

MV-infected DC Poorly Transmit Infection to T Cells. To determine if DC transmit infection to T cells a double staining (anti-NP and anti-CD3) on cytopins of cocultured APCs-T cells was performed. We observed that in cocultures with DC or monocytes, only a few T cells contained NP, in contrast to many DC or monocytes (data not shown). To quantify the percentage of each infected cell type in these cocultures, double immunofluorescence stainings with anti-NP and anti-HLA-DR or anti-NP and anti-CD3 were performed on DC-T cell cocultures and the results were analyzed by flow cytometry (Fig. 4 A). At day 3 of coculture, 38% (38-44%, $n = 5$) of DC and 9% (8.5-10%, $n = 5$) of T cells expressed NP. Similar observations were obtained from monocyte-T cell cocultures, where 40% (36-43%, $n = 5$) of monocytes versus 8% (8-10%, $n = 5$) of T cells express NP at day 3 of coculture. At the end of both cocultures, only 5-10% of the activated T cells expressed NP, whereas 45% of DC and 40% of monocytes were shown to express NP at day 5 of coculture (Fig. 4 B).

MV Infection Induces DC and T Cells to Undergo Apoptosis. In MV-infected APC-T cell cocultures, we noticed a precocious and significant decrease of viable cells, from 10^6 cells at the beginning of the cocultures to $<10^4$ at the end (only DC used as APCs are shown in Fig. 5 A). By contrast, the UV-MV weakly affected cell viability in both monocytes or DC-T cell cocultures, with the number of cells remaining nearly the same as that of uninfected APC-T cell cocultures.

The decreased cell viability in MV-infected APC-T cell cocultures correlated with the absence of thymidine incorporation by T cells even at day 1 of coculture, when most of the cells were still alive (Fig. 5 B). UV-MV partially inhibited T cell proliferation (30%) induced by DC.

To understand mechanisms by which MV decreased viable cell number and thymidine incorporation of T cells, we looked at apoptosis by the TUNEL technique. In MV-infected monocytes or DC cultures, $\sim 45\%$ of cells under-



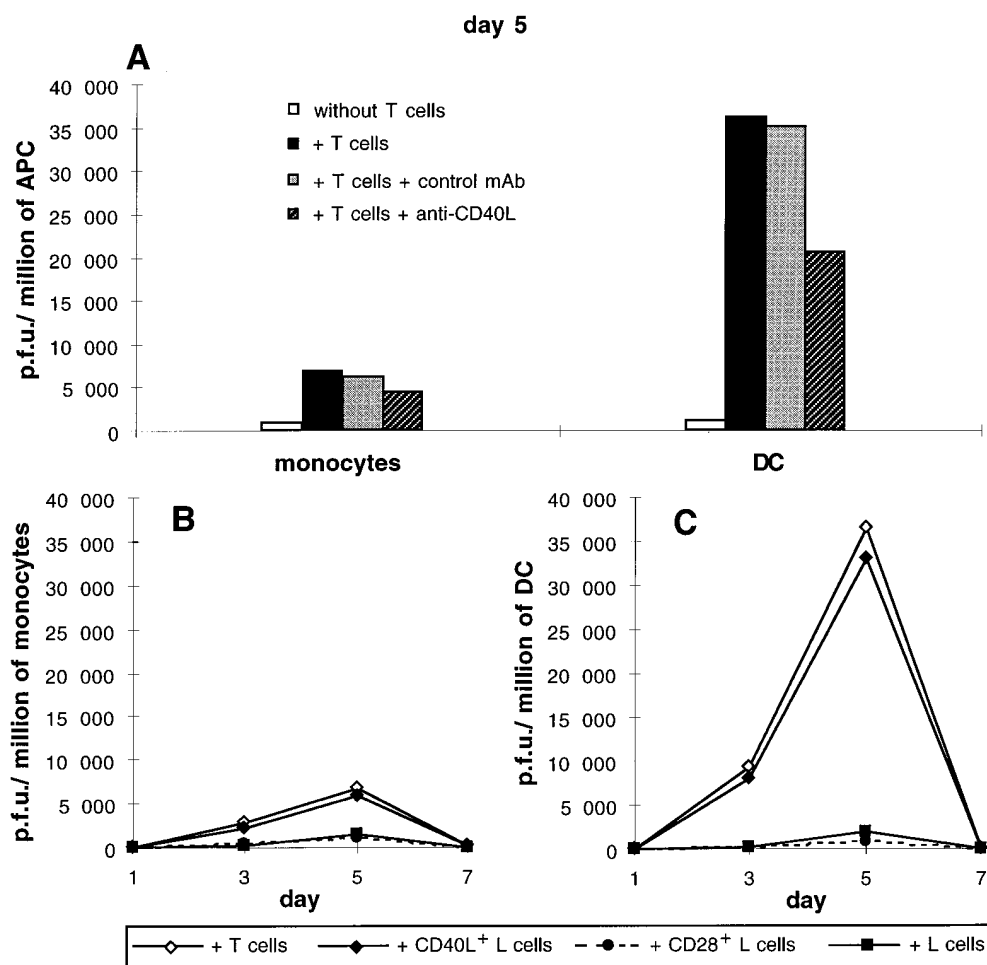


Figure 3. CD40-CD40L interaction induces a burst of MV production in MV DC-T cell cocultures. (A) Kinetics of PFU of 10⁶ MV monocytes or MV DC without or with T cells, plus anti-CD40L mAb or antibody control. Results are representative of three experiments. SD was <15%. (B and C) Kinetics of MV productions by MV monocytes (B) or MV DC (C) in the presence of CD40L⁺ L cells, CD28L⁺ L cells or T cells. Results are representative of five experiments. SD was <15% of variation.

went apoptosis at the end of the cultures, whereas in the cocultures with activated T cells, the percentage of apoptotic APCs increased rapidly to reach 50% of cells at day 1 and 90% at day 7 (only the data using DC as APCs are shown in Fig. 6 A). This apoptosis was directly linked to the infection of the cells, as no apoptotic cells were detected in APCs pulsed with UV-MV. Noticeably, T cell apoptosis reached 65% at day 1 and 95% before the end of the coculture (only the results in DC-T cell coculture are shown in Fig. 6 B). This death was largely due to the viral replication, as the apoptosis background of uninfected activated T cells cultured alone or with UV-inactivated MV pulsed APCs was <20%. In contrast to the ability of UV-MV to partially inhibit thymidine incorporation by T cells, UV-MV did not induce apoptosis of either DC or T cells. All

these data suggest that UV-MV can induce cycle arrest of T cells without inducing apoptosis.

By double staining of NP-expressing cells and of apoptotic cells, we further showed that of 98% of apoptotic T cells, only 6% had detectable NP expression (Fig. 6 C). In addition, 34 out of 82% of apoptotic APCs had detectable NP expression. In contrast, in the absence of T cells all apoptotic DC and apoptotic monocytes expressed NP.

MV Infection Downregulates IL-12 Production by CD40-activated DC. To determine if MV infection of human DC specifically downregulated IL-12 production as previously described in the case of MV-infected human monocytes (15), the kinetics of IL-12 production was measured by ELISA in culture supernatants of infected or noninfected DC. IL-12 production was detectable in culture su-

Figure 2. Immunochemical and morphological analyses of DC or DC-T cell cocultured with MV or UV-MV (A and B). At day 3, double stainings on UV-MV pulsed DC (A) and on MV-infected DC (B) were performed with anti-NP (red) and anti-HLA-DR (blue). NP can be localized in the nuclei, but is strongly expressed in the cytoplasm where the virus replicates, particularly in syncytia. (C-F). At day 3, anti-NP (red) and anti-HLA-DR (blue) stainings were performed on UV-MV DC-T cells cocultured (C and E) and on MV DC-T cells cocultured (D and F). (G and H) On CD40L⁺ L cells, syncytia were observed only with MV DC (H) and not with UV-MV DC (G). Original magnifications, A and B ×400; C and D ×200; E and F ×1000; G and H ×100.

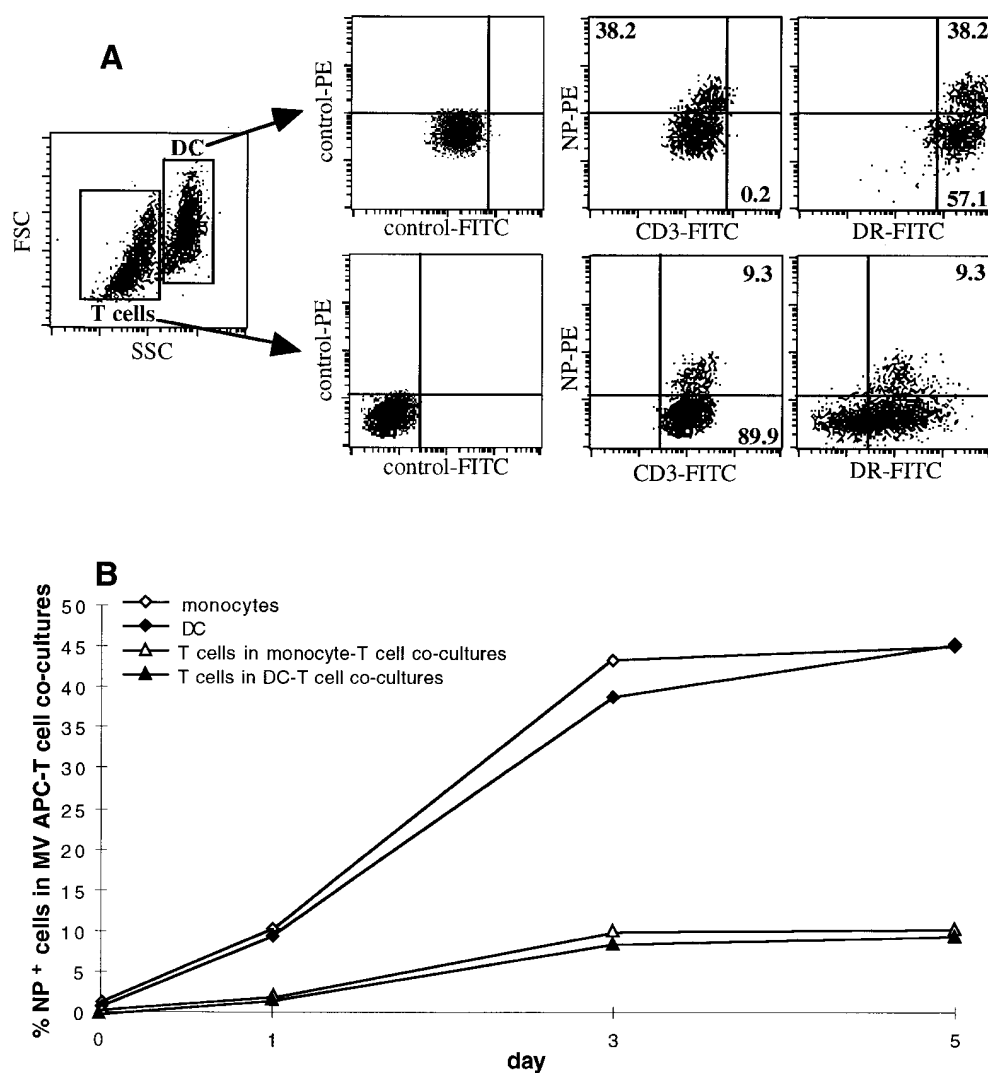


Figure 4. Percentages of NP⁺ cells in MV DC-T cell cocultures. (A) At day 3, FACS[®] analysis of NP⁺ DC and NP⁺ T cells by FITC-anti-HLA-DR/PE-anti-NP and by FITC-anti-CD3/PE-anti-NP stainings, respectively. Quad limits were positioned on the negative control which showed a higher autofluorescence in DC than in T cells. (B) Summary of the FACS[®] analysis of the NP⁺ cells in MV APC-T cell cocultured up to 5 d. Results are representative of five experiments.

pernatants from DC cocultured with either activated T cells (Fig. 7 A) or CD40L⁺ L cells (Fig. 7 B). As anti-CD40L antibody mostly abrogated IL-12-induced production, the essential role of CD40 triggering for IL-12 production by DC was confirmed (33). In these culture conditions, monocytes did not produce detectable IL-12.

The IL-12 production of DC induced by activated T cells or CD40L⁺ L cells was downregulated by adding MV (77% of the total production at day 3, 65% at day 5, 63% at day 7) and by adding UV-MV (23% at day 3, 30% at day 5, 30% at day 7) (Fig. 7, A and B). Interestingly, UV-MV, which did not induce apoptotic cell death, inhibited IL-12 production by 20–30%, suggesting that the inhibition of IL-12 production was not merely due to cell death.

Discussion

We have demonstrated the permissivity of DC to MV infection in vitro. Whereas the level of MV replication in DC is low, MV replication can be boosted by a DC-T cell interaction. Thus, DC may capture MV at its site of entry

in mucosa surfaces, then migrate to the T cell areas of lymphoid organs, where DC present immunogenic peptides to naive T lymphocytes. This DC-T cell interaction allows MV to undergo a massive replication, notably in syncytia. One of the key molecules which promotes MV replication was shown to be CD40 ligand, an important molecule for DC activation and maturation (29, 34, 35). The massive MV replication mainly occurred in CD40-activated DC, but not in T cells. However, T cell proliferation function was profoundly affected. The proliferation of T cells was more inhibited with the infectious MV than with the UV-MV. Interestingly, our data support the previous observations demonstrating that the inhibition of T cell proliferation was not the direct result of MV infection of T cells, but was due to other cell types being infected by MV within the blood (36, 37). Our study suggests that DC in the MV-infected autologous PBL may be responsible for the induction of the arrest of T cell proliferation. T cells as well as DC were induced to undergo apoptosis by MV directly (by cell infection) or indirectly (without detectable cell infection). Previous studies have also shown the immunological unre-

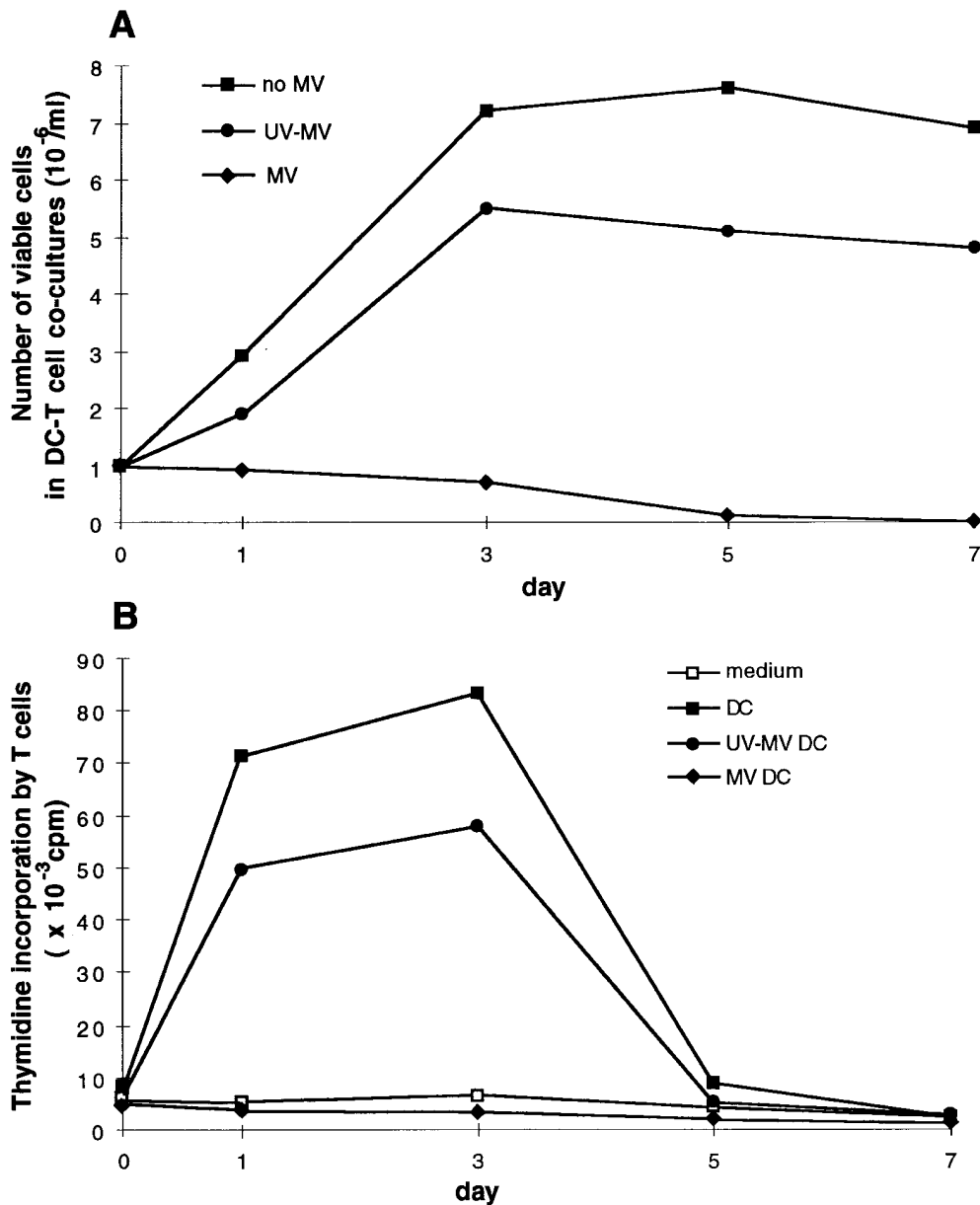


Figure 5. Number of viable cells and thymidine incorporation by T cells in DC-T cell cocultures without or with UV-MV or MV. (A) Kinetic of cell viability in DC-T cell cocultures. DC have been uninfected, pulsed with UV-MV, or infected with MV DC. (B) Kinetic of thymidine incorporation by T cells cultured alone or with uninfected DC, UV-MV DC, or MV DC. Results are representative of three experiments. SD was <10%.

sponsiveness and apoptotic cell death of T cells in MV infection (7). This induction of apoptosis may account for the mechanisms underlying the immunosuppression induced in MV patients (11, 12, 38, 39), but cannot fully explain the impairment of T cell proliferation activity, as UV-MV induces an inhibition of T cell proliferation without inducing apoptosis. It has been proposed that the mechanism of MV-induced immunosuppression may be due to the interaction of uninfected lymphocytes with MV hemagglutinin and/or fusion proteins expressed on the surface of an infected APC which then lead APCs to deliver a negative transmembrane signal to the responding T cells, which then arrests their proliferation. The ligand(s) on the uninfected T cells are unknown. We propose that members of the TNF family (for review see reference 40) may be up-

regulated on DC after MV infection, which may induce a paracrine-killing of T cells and an autocrine-killing of DC. Moreover, since apoptosis was observed in all MV-infected DC, upregulation of apoptosis inducing molecules also occurs in the absence of T cells. A small percentage of DC and T cells seems to be resistant to MV infection and to MV-induced apoptosis *in vitro*. These refractory cells may account for the recovery from measles infection. MV, both infectious and UV-inactivated, was shown to inhibit IL-12 production by CD40-activated DC. This may explain why decreases in cellular Th1 responses and increases in humoral Th2 responses in measles patients, often insufficient to defeat a viral infection, are observed.

Previous studies have shown that monocytes play a key role in MV infection and in the decrease of cell-mediated

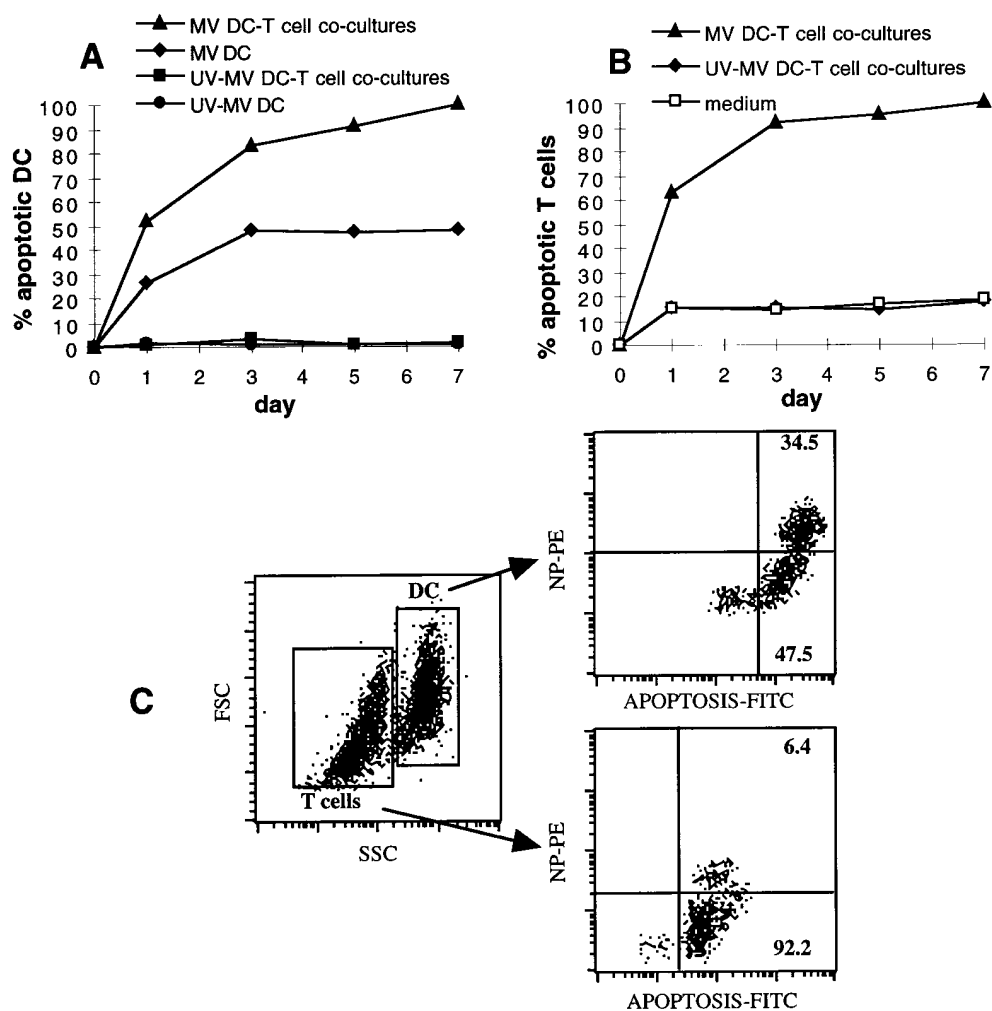


Figure 6. FACS® TUNEL analysis of DC and DC-T cell cocultures pulsed with UV-MV or MV. (A) Kinetics of apoptotic DC from UV-MV DC and MV DC cultures with or without T cells. Cells were stained with FITC-antidigoxigenin and DC were gated according to standard forward- and side-scatter values. (B) Kinetics of apoptotic T cells from DC-T cell cocultures, pulsed with UV-MV or MV. Cells were stained with FITC-antidigoxigenin and T cells were gated according to standard forward- and side-scatter values. Results are representative of three experiments. SD was <10%. (C) Apoptotic and NP⁺ cell analysis from MV DC-T cell cocultures. Cells were stained with anti-NP followed by PE-anti-mouse and FITC-antidigoxigenin. DC and T cells were gated according to standard forward- and side-scatter values. The numbers in each quadrant represent the percentages of gated DC or T cells. Quad limits were positioned on the negative control (not shown). Results are representative of five experiments.

immune responses observed during measles. In this paper, we confirm the susceptibility of monocytes to MV infection, and demonstrate the higher capacity of MV replication in CD40-activated DC. Syncytia formation could be detected in MV-infected DC or in MV-infected CD40-activated DC, but not in MV-infected monocytes or in MV-infected CD40-activated monocytes. This suggests that expression of MV hemagglutinin and/or fusion proteins as specific MV-receptor CD46 (41–43) on monocytes was not sufficient to get fusion between cells. DC might express protein(s), which is (are) absent from monocytes, but essential for syncytia formation. During acute infection, there is evidence that the cytopathic virus induces syncytia in lung and lymphoid tissues such as thymus, tonsils, lymph nodes, and spleen (9, 44). Thus, DC in these tissues might be the privileged target for the cytopathic effects of MV infection. MV infection was shown to downregulate IL-12 production by monocytes induced by LPS and IFN- γ (15). Here we showed that MV also downregulates IL-12 production by DC induced CD40-ligand. This represents a key factor contributing to the Th2 polarization observed in MV-infected patients.

MV replication in DC-T cell cocultures shared many features with HIV replication in DC-T cell cocultures: (a) only a small amount of HIV is needed to infect DC; (b) low levels of infected- DC can initiate extensive HIV-1 replication in cocultures with memory T cells, or activated T cells (45–47); (c) CD40L expressed on activated T cells may contribute to the DC-dependent HIV replication (47). However, CD28 on T cells was shown to play a role in the DC-dependent HIV replication, and in our experiments MV replication by DC was not modulated by CD28 ligation. (d) The conjugation of DC and T cell types leads to syncytia formation which may facilitate viral replication (45, 46, 48). More recently, the mechanism by which syncytia promotes HIV replication has been suggested: a syncytium formation allows active NF- κ B from the dendritic cells to conjugate with Sp-1 from the T cells, this combination being possibly involved in the acceleration of viral replication within the heterokaryon (49). It will be interesting to investigate if similar mechanisms exist in the MV-induced syncytia. (e) HIV-1-infected monocytes promote uninfected T cells to undergo apoptosis in the presence of antigen stimulation (50). (f) Finally, both HIV and MV

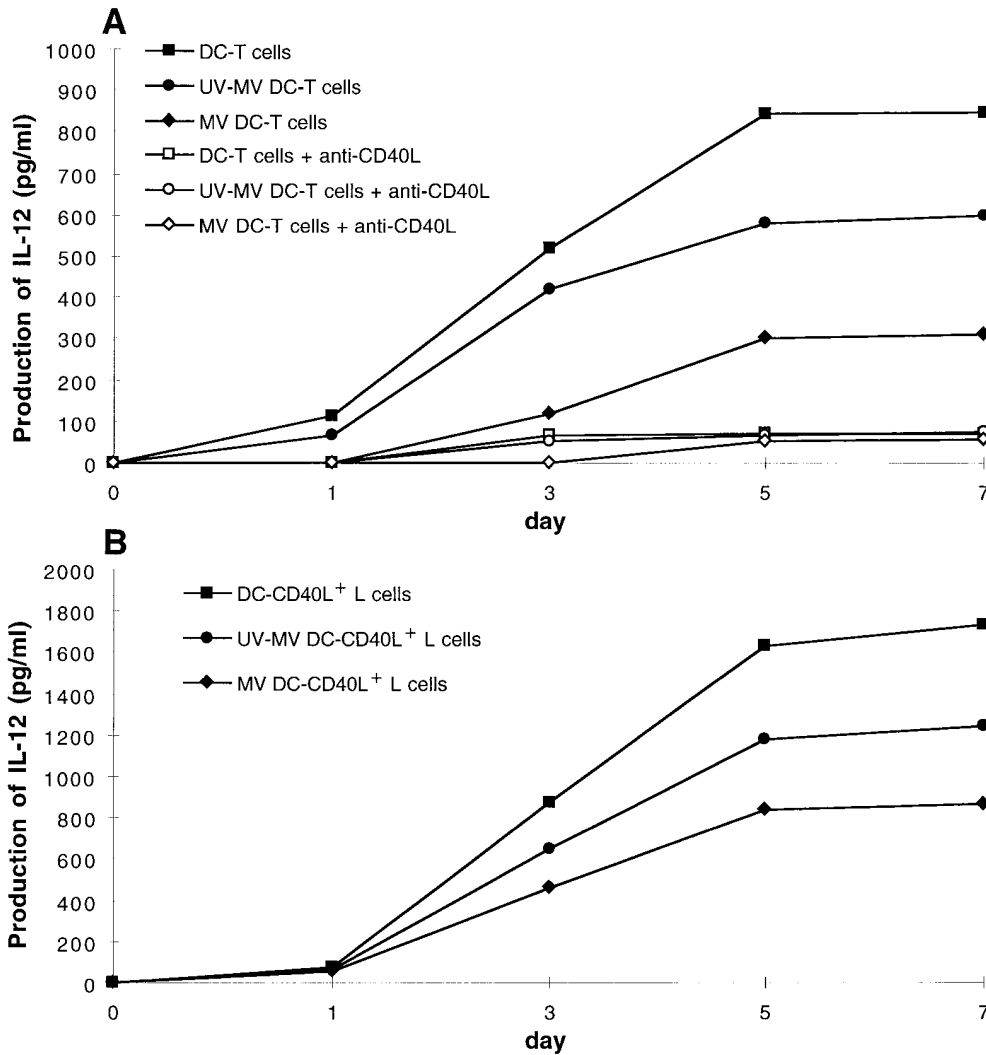


Figure 7. Kinetics of IL-12 production by CD40-activated DC. IL-12 production of DC supernatants has been measured by specific ELISA at various time points. (A) In cocultures with T cells, DC were uninfected, pulsed with UV-MV, or infected with MV in the presence or absence of mAb anti-CD40L. (B) Uninfected DC, UV-MV DC, or MV DC were cultured with CD40L⁺ L cells. Results are representative of six experiments. SD was <10%.

could induce T cell anergy, deletion, and functional polarization to Th2 (for review see reference 51).

The progression to AIDS seems due to the impairment of the immunological microenvironment to maintain the capacity for renewal of a balanced, competent Th⁻ cell

population. In this context, it will be extremely important to understand the molecular and cellular basis of how MV patients recover from initial immunosuppression occurring both at the DC and T cell levels.

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