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Measles Virus Replication in Cells of Myelomonocytic Lineage Is Dependent on Cellular Differentiation Stage

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Measles virus (MV)-infected monocytes may have a central role in virus-induced immunosuppression. Our understanding of MV replication in monocytic cells is, however, incomplete. In this work we have investigated MV replication in cells of human myelomonocytic lineage with different maturation stages in order to study the effect of cellular maturation on virus infection. MV was able to infect human bone marrow myeloid granulocyte–macrophage colony-forming cells (CFC-GM) as well as monocytes and macrophages, but the replication cycle seemed to be regulated by the maturation stage of the cells. Virus infection in CFC-GM was productive, unlike in monocytes and macrophages, where an extensive viral RNA synthesis occurred and high amounts of proteins were synthesised without a remarkable release of infectious virus. Efficiency of viral macromolecular synthesis in macrophages was comparable to that of promonocytic cell line U-937 and human epithelial cell line A549, but in contrast to macrophages the cell lines highly supported productive infection. On the other hand, chemically induced maturation of the human promyelocytic and promonocytic cell lines HL-60, THP-1, and U-937 to more mature macrophage-like forms did not markedly alter the replication cycle of MV in these cell lines. Our results showed that MV replication in myelomonocytic cells varied depending on the maturation stage of the cells. The immature myelomonocytic cells supported productive virus infection, but the maturation process lead to cellular changes that caused a restriction of MV replication cycle partly at posttranscriptional and partly at posttranslational level. The metabolic milieu of monocytes and macrophages as such was sufficient to support extensive viral macromolecular synthesis.

Key Words: measles virus; myelomonocytic cells; bone marrow.

INTRODUCTION

Measles virus (MV), a single-stranded negative-sense RNA virus, is continuously globally an important pathogen causing 1 to 2 million annual deaths, mainly in developing countries. During acute illness, MV infects peripheral blood leukocytes (Berg and Rosenthal, 1961; Sullivan *et al.,* 1975) and monocytes are the major target cells (Salonen *et al.,* 1988; Esolen *et al.,* 1993). Infection causes strong immunosuppression with currently rather poorly known mechanisms. Infected monocytes may have a central role in the induction of immunosuppression. MV infection in monocytes changes many of their functions, e.g., by increasing production of interleukin-1β and reducing levels of tumour necrosis factor-α and interleukin-12 (Leopardi *et al.,* 1992; Ward *et al.,* 1991; Karp *et al.,* 1996). The expression of MHC class II molecules and the antigen-presenting function of HLA-DR, -DQ, and -DP molecules are enhanced in MV-infected monocytes *in vitro,* but no change is detected in infected promonocytic cell line THP-1, indicating that activation and/or maturation stage of the cells may play a pivotal role in virus-induced events (Leopardi *et al.,* 1993).

Besides disturbances caused by MV infection in immunological functions, infected cells of immune system can transport the virus to various target organs. MV replication in primary monocytes is highly restricted (Vainionpää *et al.,* 1991; Karp *et al.,* 1996), whereas immature cord blood monocytes from neonates support productive virus infection (Sullivan *et al.,* 1975). Moreover, the report by Bashle and co-workers (1985) suggests that MV replication may vary with the stage of differentiation or maturation of the cells. They have shown MV antigens in osteoclasts from patients with Paget's disease. Osteoclasts originate from bone marrow via mononuclear cells and have characteristics similar to macrophages. MV antigens were detected in both nuclei and cytoplasms of osteoclasts. This kind of intracellular distribution of MV antigens is known to be typical for persistently MV-infected cells (Norrby *et al.,* 1982; Chui *et al.,* 1986). Although an increasing number of studies indicate an important role of infected monocytes in MV immunopathogenesis, our knowledge about virus replication in myelomonocytic cells is limited. It is therefore of interest to analyse MV infection in more details in myelomonocytic cells with different maturation stages

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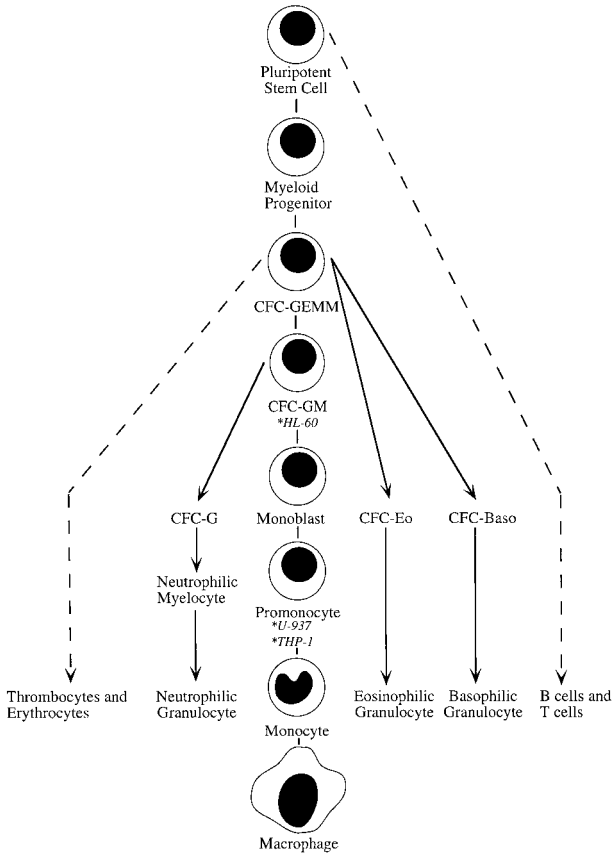

FIG. 1. Maturation and differentiation of myelomonocytic cells.

and study if host cell factors induced during maturation can modulate virus replication cycle.

To understand better MV infection in monocytic cells we have analysed MV replication in myelomonocytic cells with different maturation stages, from human bone marrow granulocyte/macrophage progenitors to monocytes and macrophages, as well as in promyelocytic and promonocytic cell lines HL-60, THP-1, and U-937. MV replication was productive in immature cells and restricted in mature monocytes/macrophages and the restriction occurred both at posttranscriptional and at posttranslational levels.

RESULTS

Maturation and differentiation of the cells of myelomonocytic lineage and the maturation stages of the promonocytic and promyelocytic cell lines THP-1, U-937, and HL-60 used in this study are shown in Fig. 1.

Myelomonocytic progenitors support productive MV replication

The mononuclear bone marrow cells divide approximately six times during the culturing period of 14 days, and granulocyte–macrophage colony-stimulating factor (GM-CSF) present in the culture medium supports exclusively the growth of only granulocyte–macrophage colony-forming cells (CFC-GM). Therefore, each colony is a product of one single progenitor cell and contains a mixture of original-type progenitor cells and more or less differentiated but still very premature cells of the myeloid lineage.

In contrast to mature monocytes, MV was able to replicate productively in progenitor cells of myelomonocytic lineage. The amount of infectious virus in culture medium increased from 8×10^1 PFU/ml (representing the rest of inoculum virus in medium after washings) to 3×10^3 PFU/ml (3 days p.i.) being at the same level still at day 5 p.i. Figure 2 shows a one-step growth curve of MV in CFC-GM. Immunofluorescent staining of the colonies showed sporadic MV antigen-positive cells, suggesting that infection was limited to a certain cell type (data not shown).

MV replication in monocytes/macrophages is restricted

We have earlier shown that MV replication in monocytes is incomplete. 12-*O*-Tetradecanoylphorbol 13-acetate (TPA) and Ca^{2+}-ionophore, which enhance protein kinase C activity and change a nonproductive MV replication to a productive one in peripheral blood mononuclear cells (PBMC), could not induce production of infectious virus in monocytes (Vainionpää *et al.,* 1991). In this study we examined whether differentiation of monocytes by IL-3 and/or GM-CSF would increase MV infection in monocytes/macrophages. For these experiments, monocytes were infected with MV, washed, and incubated for 3 days, after which maintenance medium was replaced by medium containing various concentrations of IL-3 (1, 10, or 25 IU/ml) and/or GM-CSF (10, 25, or 50 IU/ml). Incubation was continued for further 3 days and the specimens were collected for infectivity titration assay. Although more than 70% of monocytes in cultures contained intracellular MV proteins when examined by im-

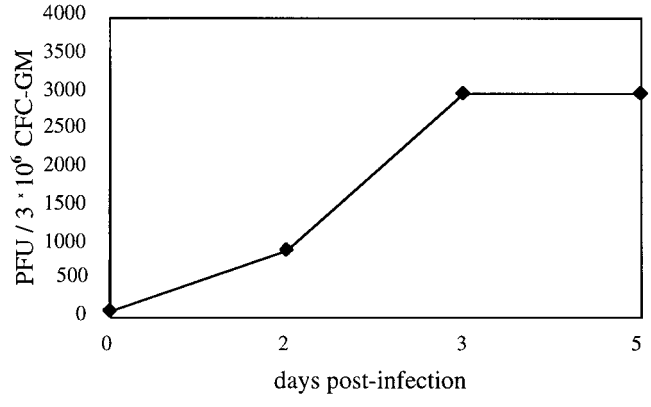

FIG. 2. One-step growth curve of measles virus in granulocyte–macrophage colony-forming cells (CFC-GM) quantified from the culture medium by plaque titration assay in Vero cells.

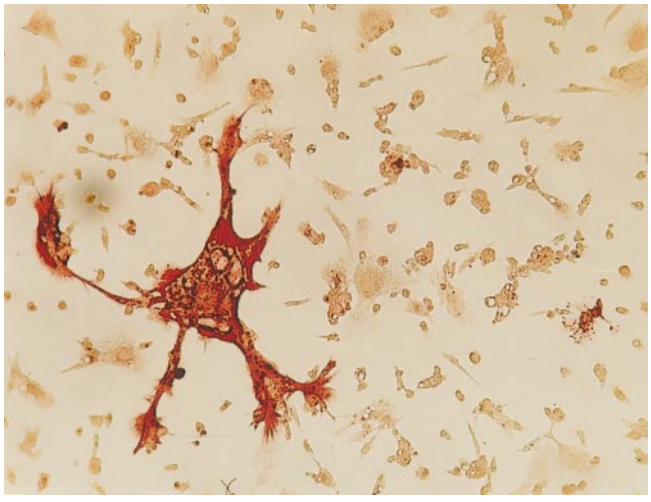

FIG. 3. MV infection of macrophages. Monocytes were infected with MV and then incubated in mitogen-stimulated medium for 3 days, after which the cells were fixed with 75% acetone and stained with the rabbit anti-measles virus serum and the HRP-conjugated goat anti-rabbit IgG. The figure shows one MV-infected, multinucleated macrophage and several smaller uninfected and infected mononucleated macrophages.

munofluorescent staining, neither the individual cytokines IL-3 or GM-CSF nor their mixture were able to increase MV replication (data not shown).

In order to further study the effect of maturation of monocytes to macrophages on their capacity to support MV replication, monocytes were infected, incubated for 3 days, and the maintenance medium was replaced by "mitogen-stimulated" medium or by medium from unstimulated PBMC culture. After 3 days the cells treated with mitogen-stimulated medium appeared as macrophage-like cells, and most of them were strongly positive for MV antigens when stained by the rabbit anti-measles virus antibodies. The cultures also contained MV antigen-positive multinucleated giant cells, one of which has been shown in Fig. 3. Maturation of infected monocytes to macrophages did not, however, change a restricted virus replication to a productive stage. Freezing and thawing did not have any effect on the release of infectious virus from macrophages either.

MV replication in monocyte-derived macrophage cultures was analysed in more detail by Northern and Western blotting techniques as well as by the infectivity titration assay. MV replication in the promonocytic cell line U-937 and in the epithelial cell line A549 was analysed for comparison. The macrophage cultures were maturated in macrophage SFM medium for 14 days as described under Materials and Methods. Figure 4 shows that virus RNA synthesis was more effective in macrophage cultures than the RNA synthesis in U-937 cells and comparable to the RNA synthesis in A549 cells, which are known to productively support MV replication. Viral proteins in macrophages were detected as well, indicating ongoing virus protein synthesis, although the protein amount was much lower than the proteins in the cell lines (Fig. 5). No release of infectious virus from macrophage cultures occurred, however. In contrast, virus replication in U-937 cells as well as in A549 cells was highly productive, the virus titres being 2×10^5 PFU/ml and 7×10^4 PFU/ml, respectively. These results showed that the metabolic milieu of monocytes/macrophages support MV macromolecule synthesis and the inhibition of effective virus production occurs at both posttranscriptional and posttranslational levels.

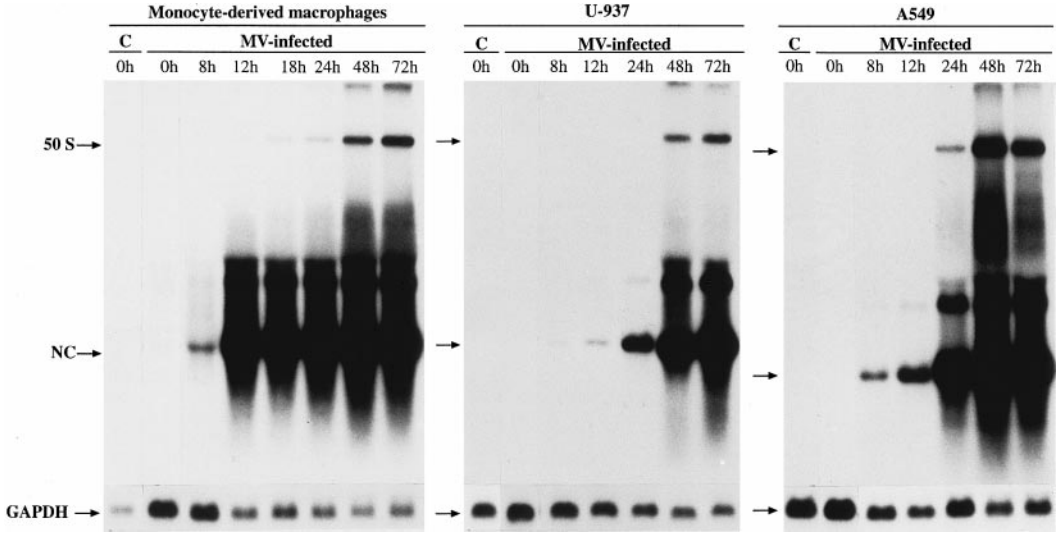

FIG. 4. Northern blotting analysis of total RNA in MV-infected monocyte-derived macrophages, U-937, and A549 cells. The specimens were collected at the times indicated. The filters were hybridised with the MV NC cDNA probe as well as with the GAPDH cDNA, which was used as an internal standard. The positions of the MV genomic size RNA 50S and NC are marked by the arrows. C, total RNA from uninfected cells.

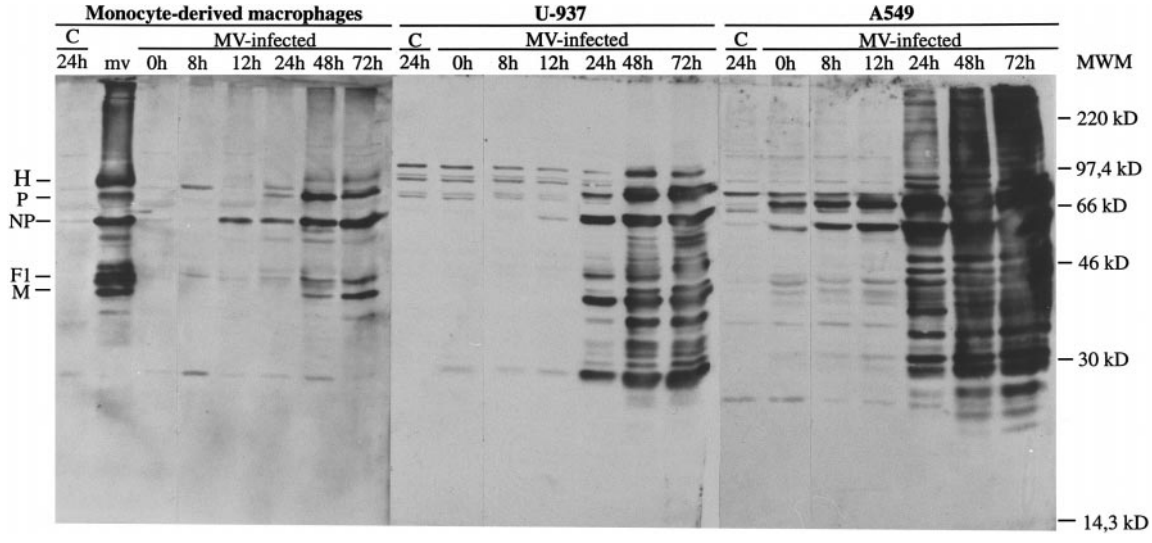

FIG. 5. Western immunoblot detection of MV proteins in monocyte-derived macrophages, U-937, and A549 cells. The specimens were collected at the times indicated, separated by SDS–PAGE, blotted onto nitrocellulose membrane, incubated with the rabbit anti-measles virus serum and the anti-rabbit HRP-conjugate, and detected by enhanced chemiluminescence. The positions of MV structural proteins are indicated at the left and the molecular weight markers at the right. mv, inoculum virus. C, uninfected cells.

MV infection in promyelocytic and promonocytic cell lines

For comparison, we studied MV replication also in the human promyelocytic and promonocytic cell lines HL-60, THP-1, and U-937. These cell lines, each of which represents a different stage of maturation, are widely used as *in vitro* models to study interactions between viruses and monocytes because they display a number of monocytic characteristics. They can also be induced to mature with phorbol esters such as TPA to more mature macrophage-like cells. HL-60 cells are closely related to early progenitor cells. They have bilinear differentiation potential and they can be chemically induced to differentiate to either granulocytic or monocytic cells. THP-1 and U-937 cell lines are from the monocyte/macrophage lineage and U-937 cells represent a later maturation stage. Chemically induced maturation has been reported to change the permissivity of these cell lines for replication of many other viruses (Roivainen and Hovi, 1989; Tenney and Morahan, 1991; Weinshenker *et al.,* 1988).

All three cell lines supported viral RNA synthesis to a similar extent (Fig. 6) when followed by spot hybridisation and measuring the radioactivity of the spots by liquid scintillation counting. However, the amount of released infectious virus did not correlate with the amount of viral RNA. The virus release was most effective in the promonocytic cell lines THP-1 (1×10^5 $TCID_{50}$) and U-937 (1×10^5 $TCID_{50}$). In contrast, the promyelocytic cell line HL-60 supported virus release only at a minimal level (5×10^1 $TCID_{50}$).

Because MV replication in PBMC is dependent on cellular activation stage (Lucas *et al.,* 1978; Hyypiä *et al.,* 1985), we wanted to compare the proliferation capacity of these cell lines by measuring their DNA synthesis by [^{3}H]thymidine incorporation. As shown in Fig. 7, THP-1 and U-937 cells were metabolically more active than HL-60 cells, and ongoing MV replication in these cells caused strong suppression of cellular DNA synthesis which was most evident in U-937 cells.

TPA is known to cause maturation of HL-60, U-937, and THP-1 cells (Tsuchiya *et al.,* 1982). In order to study the effect of maturation on MV replication, the cell lines were

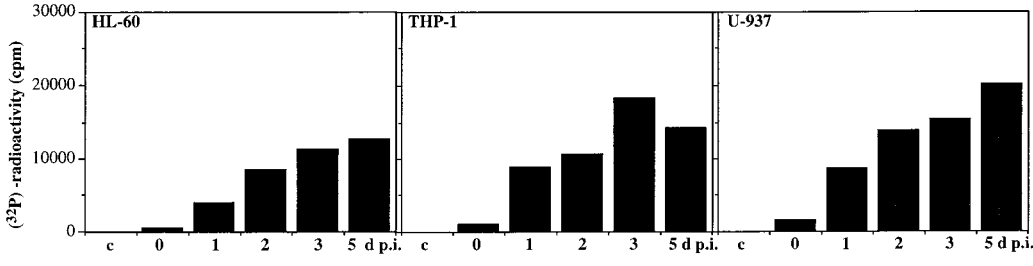

FIG. 6. MV-specific RNA synthesis in the promyelocytic and promonocytic cell lines HL-60, THP-1, and U-937 analysed by spot hybridisation and by measuring the radioactivity of the spots by liquid scintillation counting.

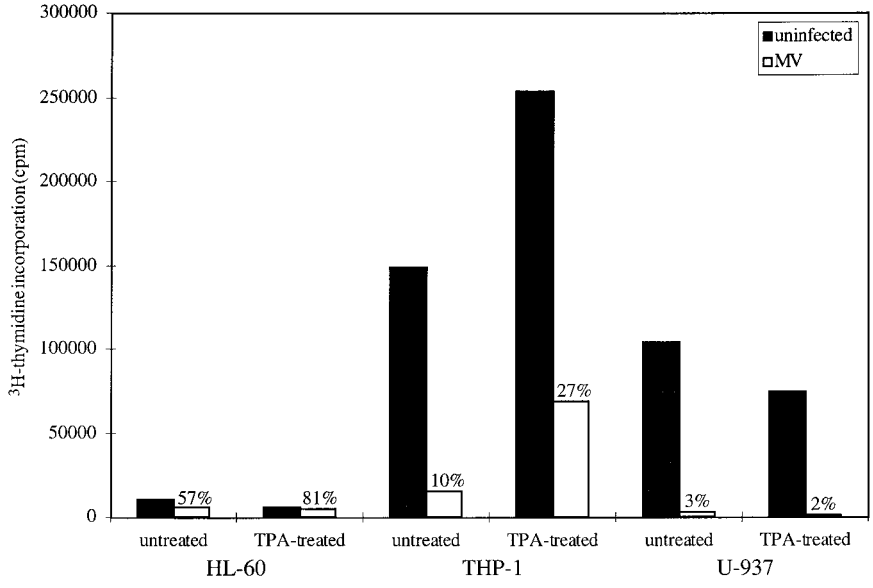

FIG. 7. DNA synthesis, as measured by [^{3}H]thymidine incorporation, in MV-infected and uninfected monocytic cell lines, which were either untreated or pretreated with TPA (1.0 nM) for 24 h before infection. The mean cpm (counts per minute) values of three parallel cultures are shown. The cpm values of MV-infected samples relative to corresponding uninfected samples are presented in percentages.

treated with 1 nM of TPA for 24 h before infection. The changes caused by maturation on cell proliferation were followed by measuring DNA synthesis by [^{3}H]thymidine incorporation (Fig. 7). TPA stimulated the proliferation of THP-1 cells, but caused suppression of DNA synthesis in HL-60 and U-937 cells. No clear-cut effect of cellular maturation on virus RNA synthesis as determined by spot hybridisation or on virus production was detected (data not shown). Our results showed that although TPA-induced maturation caused changes in cellular activity, no similar decrease/increase was observed in virus replication, indicating that MV replication did not depend entirely on the metabolic activity of the host cell.

DISCUSSION

Our results showed that human bone marrow progenitor cells were susceptible to productive MV replication. During the maturation of myelomonocytic cells some changes occurred, which lead to restriction of MV infection. Monocytes/macrophages supported extensive viral RNA and protein synthesis, but no clear-cut release of infectious virus was observed.

Infected monocytes may have a central role as mediators of immunosuppression, which is most probably a multifactorial event. On one hand, active virus replication is needed for suppression, because UV-inactivated virus is able to suppress cell proliferation in a much less extent than infectious virus does. On the other hand, interaction of MV glycoproteins with the surface of uninfected PBMC is sufficient to induce immunosuppression (Schlender *et al.,* 1996). Evidently cell cycle arrest of infected B- and T-lymphocytes is also partly responsible for suppression (McChesney *et al.,* 1987, 1988). MV is known to cause many cytokine dysfunctions of monocytes (Griffin and Ward, 1993), e.g., down-regulation of IL-12 (Karp *et al.,* 1996), which is known to be critical for the generation of cell-mediated immunity. This down-regulation was induced also by UV-inactivated virus. One obvious mechanism involved in immunosuppression is apoptosis observed in monocytes, dendritic cells, and T-lymphocytes in MV-infected cultures. MV-infected dendritic cells have been shown to induce apoptosis also in uninfected T-lymphocytes (Fugier-Vivier *et al.,* 1997).

As described above, an increasing amount of information suggests the central role of monocytic cells in MV immunopathogenesis, but much less is known about virus replication in myelomonocytic cells. Sullivan *et al.* (1975) have reported that the immature cord blood monocytes from neonates support a complete replication cycle of MV, and because a considerable proportion of monocytes in circulation can be immature, MV infection in these cells can contribute to the spreading of infection. An interesting question is whether MV can infect bone marrow progenitor cells. MV is known to cause life-long immunity, and it has been proposed that the persistence of the virus in bone marrow could be a reason for prolonged antibody production after primary infection. In this work we demonstrated that MV was able to infect bone marrow myeloid progenitor cells *in vitro,* and CFC-GM supported productive MV infection. Bone marrow cells are known to be affected in a number of different virus infections. For instance, human parvovirus B19 can cause aplastic crisis in patients with sickle cell anaemia (Pattison *et al.,* 1981), cytomegalovirus in-

fects both stromal and hematopoietic progenitor cells (Maciejewski *et al.,* 1992), and human herpes virus 6 (Knox and Carrigan, 1992) and dengue virus (Nakao *et al.,* 1989) infect bone marrow cells. To our knowledge this is the first time when MV has been shown to infect and replicate in human bone marrow progenitor cells.

There is variation in reports concerning MV replication in monocytes. Joseph *et al.* (1975) have described productive MV replication in monocytes, whereas we and others have shown that in mature monocytes/macrophages MV replication is highly restricted, and stimulation with various extracellular mitogens, which activate different biochemical pathways, does not activate the silent infection to a productive one (Vainionpää *et al.,* 1991; Karp *et al.,* 1996). This variation might be caused by different factors, e.g., maturation stage of monocytes. Also the susceptibility of monocytes from different individuals can vary.

In this report we have shown that the metabolic milieu of monocytes/macrophages as such could support MV macromolecular synthesis, because extensive viral RNA and protein synthesis occurred. The active virus protein synthesis, without release of infectious virus, in monocytes/macrophages may lead to accumulation of virus glycoproteins on cell surface, and this phenomenon could be an important factor in monocyte-mediated immunosuppressive events. Also the report by Bashle and co-workers (1985), describing persistent-type MV infection in osteoclasts, suggests restriction of MV replication in mature cells of the same myelomonocytic lineage. Cirino and co-workers (1993) have reported that respiratory syncytial virus (RSV), a member in the family of Paramyxoviridae, replicates productively in freshly isolated alveolar macrophages, but *in vitro* differentiation of monocytes into macrophages results in a significant, time-dependent decrease in production of infectious virus. The mechanisms by which cellular differentiation restricts MV and RSV replication are still unknown.

As a conclusion, our results suggest that the maturation stage of myelomonocytic cells may have an important role in the pathogenesis of measles. On one hand, immature monocytic cells can support productive virus replication, which can lead to the dissemination of the virus in the body. On the other hand, mature monocytes/macrophages support extensive virus RNA and protein synthesis, without release of infectious virus. These cells containing high amounts of virus proteins can be responsible for other dysfunctions, such as immunosuppression occurring in MV pathogenesis.

MATERIALS AND METHODS

Virus

A wild-type measles virus (Halonen-strain, Vainionpää *et al.,* 1978) with a high infectivity titre ($>1 \times 10^7$ PFU/ml) was used throughout the study. The inoculum virus was propagated in Vero cells. For RNA and protein isolation the cells were infected at a multiplicity of infection (m.o.i.) of 5. In all the other works cells were infected at a m.o.i. of 1. After the adsorption time of 60 min those cultures later checked for virus production were thoroughly washed, and fresh maintenance medium was added.

Bone marrow progenitor cells

CFC-GM cultures were prepared as described earlier by Vuorinen *et al.* (1996). Briefly, mononuclear cells were obtained by the COBE 2991 Model I blood cell processor (COBE) from Ficoll–Paque (Pharmacia, Uppsala, Sweden)-separated heparinised bone marrow collected from autologous transplantation patients (disease-free at the time of the collection). The *in vitro* colonies of hematopoietic progenitors were cultured by the methyl cellulose technique originally developed by Pike and Robinson (1970) with the modification of Guilbert and Iscove (1976). Mononuclear bone marrow cells (2×10^5/ml) were mixed with culture medium containing 1% methyl cellulose, 20% foetal bovine serum (FBS, HyClone), 1% delipidated and deionised bovine serum albumin (Sigma, Cell Culture), 1×10^{-4} M β-mercaptoethanol, and 0.5 mg/ml fully iron-saturated human transferrin (Behringwerke) in Iscove's Modified Dulbecco's minimum essential medium (Gibco) and added to plastic Petri dishes. CFC-GM were cultured in the presence of GM-CSF (Leucomax, Sandoz, Schering-Plough), which supports the growth of only granulocytic and monocytic lineage cells. The plates were incubated for 14 days at 37°C in a fully humidified atmosphere with 5% CO_2.

The propagation of the bone marrow cultures was performed by Central Laboratory, Department of Haematology, Turku University Central Hospital to check the viability of autologous bone marrow transplantation material. The propagation took 2 weeks after which this anonymous waste material could be used for our purpose.

For virus infection the monocytoid colonies were picked under a light microscope, infected with MV (1 m.o.i.), washed, and suspended into the culture medium (without methyl cellulose) as described above.

Other cells and cell lines

Human peripheral blood puffy coat fractions of healthy blood donors were obtained from The Finnish Red Cross Blood Transfusion Service, Turku. To isolate mononuclear cells, the puffy coat cells were centrifuged at 1600 rpm for 45 min through a Ficoll–Paque cushion. The monocytes were enriched by adherence to polystyrene plastic plates, and nonadherent cells were removed by washing with Hanks' balanced salt solution. The cell preparations contained more than 90% monocytes as estimated by their light scattering properties in fluorescence-activated cell sorter analysis. The cells were

maintained at 37°C with 5% CO_2 in RPMI 1640 medium (Gibco) supplemented with 10% human AB serum; in MV-infected cultures 10% FBS was used instead of human serum.

Monocyte-derived macrophages were cultured from monocytes (isolated as described above) in macrophage SFM medium (Gibco) containing 10 ng/ml of GM-CSF for 14 days, during which period the medium was replaced every second day by fresh medium. Maturation of monocytes to macrophages was also done by incubating the MV-infected monocytes in mitogen-stimulated medium, which was a supernatant from concanavalin A (Con A)-treated uninfected PBMC. For preparation of such a supernatant, uninfected PBMC were treated with Con A (10 μg/ml) overnight, after which Con A was washed out with medium containing 0.3 M α-methyl-D-mannoside. Incubation was continued for 2 days, and the cells were pelleted and the supernatant was used as mitogen-stimulated medium. Maturation of the monocytes was detected based on morphology.

The human promyelocytic cell line HL-60 (ATCC CCL-240) and the promonocytic cell lines THP-1 (ATCC TIB-202) and U-937 (ATCC CRL-1593) were cultured in RPMI 1640 medium supplemented with 10% FBS. The maturation of the cell lines was induced by treatment with 1.0 nM of TPA (Sigma, St. Louis, MO) overnight. The human epithelial cell line A549 (ATCC CCC-185) was maintained in Ham's F-12 medium.

Assays for virus infectivity

The amount of infectious virus was determined by incubation of serial 10-fold dilutions of supernatants from infected cells on confluent monolayers of Vero cells. Each dilution was assayed in duplicate. After 5 to 7 days, the virus titre was determined after either a standard plaque counting (Vainionpää *et al.,* 1978) or a reading of the $TCID_{50}$ by light microscopy.

Immunostaining of cells

Immunoperoxidase staining of macrophages was done as described by Waris *et al.* (1990). Briefly, the cells were fixed on polystyrene plastic plates with cold 75% acetone and incubated first with the rabbit anti-measles virus serum and then with the horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Bio-Rad Laboratories; diluted 1/400). 3-Amino-9-ethylcarbazole dissolved in dimethylformamide was used as chromogen.

Nucleic acid hybridisation

A cDNA clone composed of 1.6 kb of MV nucleoprotein mRNA in a pBR322 vector was used as a probe. Labelling of the probe, treatment of the cells, and hybridisation procedures were done as described by Hyypiä *et al.* (1985).

RNA isolation and Northern blotting

For RNA analysis, the specimens (10×10^6 cells) were harvested at indicated times. Total cellular RNA was isolated by guanidium isothiocyanate lysis followed by CsCl centrifugation 35,000 rpm overnight at 15°C (Chirgvin *et al.,* 1979). Equal amounts of RNA (20 μg) were electrophoresed in a 0.8% formaldehyde–agarose gel, transferred to a Zeta-Probe GT genomic tested blotting membrane (Bio-Rad Laboratories, CA) and hybridised with MV nucleocapsid-specific cDNA probe (a gift of Dr. T. Wong, University of Washington, Seattle, WA), labelled with [α-^{32}P]dCTP (Amersham) by a random primed DNA labelling kit. Determination of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA by a cDNA probe (Fort *et al.,* 1985) was used as an internal control.

Immunoblotting

For virus protein analysis, the cells were lysed in RIPA buffer (50 mM Tris–HCl buffer, pH 8.0, containing 1% NP-40, 0.4% Na-deoxycholate, 0.1% SDS, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml of leupeptin and aprotinin) and centrifuged 14,000 rpm for 30 min to remove insoluble material. Protein concentrations were estimated by Bio-Rad protein assay kit. The proteins (100 μg/lane) were separated by 10% SDS–PAGE, transferred electrically by Semi Dry Blot Pegasus (PHASE GmbH, Germany) onto Schleicher & Schuell nitrocellulose filter. After the blocking with 5% milk, the filters were incubated with the rabbit anti-measles virus antibodies (dilution 1:125 in phosphate-buffered saline containing 1% Triton X-100 and 5% milk powder) for 3 h at 37°C and then with the anti-rabbit HRP-conjugate. The bands were visualised by ECL chemiluminescence system (Amersham).

Cell proliferation assay

DNA synthesis of the untreated and TPA-maturated cell lines was measured by [^{3}H]thymidine incorporation. For maturation, the cells were treated with TPA at a concentration of 1 nM for 24 h before the infection and cultivated at a density of 1×10^5 cells/200 μl. [^{3}H]thymidine (Amersham; 0.5 μCi per well) was added 20 h before harvesting. The cells were harvested 4 days postinfection with a multichannel semiautomatic cell harvester (Skatron), and the radioactivity was measured in a 1217 Rackbeta liquid scintillation counter (Wallac, LKB).

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