Respiratory Virus Infection of Peripheral Blood Monocytes: Correlation with Ageing of Cells and Interferon Production in vitro

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SUMMARY

The ability of respiratory syncytial virus (RSV) and parainfluenza virus type 3 (PIV3) to replicate in peripheral blood monocytes cultured in vitro for 1, 2, 4 or 7 days prior to infection was investigated. Inoculation of 1-day old monocytes produced at least tenfold less new virus than infection of the older, more macrophage-like cells for both viruses. PIV3 induced extensive syncytium formation, whereas RSV caused a cytopathic effect manifest by increased rounding of the cells with minimal syncytium formation. Supernatants of infected monocytes were assayed for human interferon-α (HuIFN-α) in an attempt to explain the restricted viral replication in the youngest monocytes. In PIV3-infected cells, HuIFN-α production was inversely correlated with new virus formation. Monocytes infected after 1 day in culture produced 800 IU/ml of HuIFN-α; the older cells produced 100 to 200 IU/ml. In contrast, monocytes infected on day 1 with RSV produced minimal amounts (1.5 IU/ml) of HuIFN-α. Increasing amounts of HuIFN-α were detected in cells infected with RSV after 2, 4 or 7 days in culture, reaching a maximum of 400 IU/ml on day 7. Further investigation of the apparent restriction of replication in young monocyte cultures may be helpful in understanding the pathogenesis of these respiratory infections.

Respiratory syncytial virus (RSV) and parainfluenza virus type 3 (PIV3) are major causes of acute lower respiratory tract illness in infants as well as being responsible for upper respiratory infections in people of all ages (Glezen et al., 1984; Mufson et al., 1970). Both viruses can produce similar respiratory syndromes and can reinfect infants resulting in significant respiratory illness even after a brief recovery period. RSV is a poor inducer of interferon in vivo in nasal secretions whereas parainfluenza viruses can induce a significant local interferon response (Hall et al., 1978, 1981; McIntosh, 1978).

The monocyte/macrophage is an integral part of the cellular immune response to viral infection (Becker, 1984). These cells can be involved in responses to respiratory viral infection as circulating monocytes and/or tissue macrophages. Although their exact role in the response to respiratory viral infection is not known, specific cell-mediated immunity to both RSV and PIV3 has been documented (Roberts, 1982; McIntosh et al., 1979). Studies in vitro have shown that Sendai virus inhibits the ability of peripheral blood monocytes to enhance lymphocyte transformation to phytohaemagglutinin and leads to significant interferon α (HuIFN-α) production by monocytes, whereas RSV did not affect either of these monocyte functions (Chonmaitree et al., 1981; Morahan et al., 1985). Despite this lack of effect on monocytes, RSV has recently been shown to be capable of infecting human peripheral blood monocytes in vitro.

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and RSV antigen has been found in circulating monocytes of infants with RSV disease (Domurat et al., 1985).

We have productively infected peripheral blood monocytes with RSV or PIV3 in vitro and measured both new virus and HuIFN-α production.

Peripheral venous blood from healthy adult volunteers was sedimented over Ficoll-Hypaque (Pharmacia) (Boyum, 1968). Mononuclear cells were collected and washed three times with Hanks' balanced salt solution (HBSS). Viability was assessed by trypan blue (0-04%) exclusion. The cell concentration was adjusted to 10⁷ to 2 x 10⁷ cells/ml in RPMI 1640 supplemented with 20% autologous human serum. Samples (0-2 ml) were applied twice to siliconized (Siliclad, Clay Adams, Parsippany, N.J., U.S.A.) coverslips (15 mm, Propper Manufacturing Co., Piscataway, N.J., U.S.A.) and incubated for 1 h each time at 37 °C. Between and after the incubations the coverslips were rinsed in HBSS and then incubated in 24-well Linbro dishes (Flow Laboratories) with 0-5 ml RPMI 1640 and 20% autologous serum. This was designated day zero. On day 1 all coverslips were rinsed in HBSS and incubated with RPMI 1640 with 20% foetal calf serum (FCS). Cells were > 90% monocytes on days 1 and 7 as assessed by non-specific esterase staining (α-naphthyl acetate esterase; Sigma). Cells were infected with virus as described below on days 1, 2, 4 or 7 after collection.

RSV (Long strain) and PIV3 (strain C243; American Type Culture Collection) were grown in HEp-2 cells, purified and concentrated by ultracentrifugation using a modification of the method of Fernie & Gerin (1980). For both viruses a peak titre of > 1 x 10⁶ p.f.u./ml was obtained from gradients at a density of 1.18 g/ml. Uninfected HEp-2 cell culture fluid was processed similarly for use in mock infections.

Cells were infected at an m.o.i. of 0-5 at 37 °C. Cells were then washed with HBSS and incubated in 0-5 ml RPMI 1640 with 20% FCS. Supernatants were collected at 24 h intervals, the cells washed with HBSS, and reincubated with 0-5 ml fresh medium for 96 h. After 96 h the coverslips were removed, fixed for 10 min in cold acetone, and stored frozen at −20 °C. Each time point was run in duplicate. The fixed monocyte cell layers were stained using bovine polyclonal antisera to either RSV or PIV3 (Burroughs Wellcome, Beckenham, U.K.) at a 1 : 8 dilution and fluorescein-coupled goat anti-bovine IgG (Kirkgaard and Perry, Gaithersburg, Md., U.S.A.) at a 1 : 40 dilution.

The supernatants from each time period were assayed in duplicate for viral infectivity by a plaque assay technique using the addition of 0-025 ml of serial tenfold dilutions to HEp-2 cells (10⁵ cells/well) in 96-well plates (Corning no. 25860).

Enzyme-linked immunosorbent assay (ELISA) for detection of RSV antigen was performed on the monocyte supernatants in microtitre plates using an indirect method as previously described (Hendry & McIntosh, 1982).

HuIFN-α was measured by a modification of the vesicular stomatitis virus (VSV) plaque-reduction technique of Havell & Vilcek (1982) using embryonic bovine tracheal cells. The final concentration of units of HuIFN-α per ml of sample was determined by comparison to a known HuIFN-α standard (50 international units/ml; New York Blood Center, lot 107; compliments of Dr Michael Wiebe) which was included in each assay. RSV and PIV3 were added to the assay with HuIFN-α standards and did not affect the results. The specificity of the assay was tested by blocking the activity of interferon with a rabbit anti-HuIFN-α antibody (Enzo Biochemicals, New York, N.Y., U.S.A.).

Adherent monocytes exposed to RSV or PIV3 produced new infectious virus and, for RSV, viral antigen within 48 h of infection as detected by plaque assay and ELISA of cell supernatants (Fig. 1). The use of purified and unpurified virus preparations for the initial infection produced identical results (data not shown). For both viruses the youngest cells (1 day in culture after separation) always produced at least tenfold less infectious virus than older cells. The number of days in culture at which these older cells produced the most infectious virus varied in different experiments.

PIV3 infection produced significant cytopathic changes with extensive syncytium formation occurring within 24 h of infection regardless of the time period in tissue culture prior to infection. In contrast RSV caused a c.p.e. manifest by rounding of the cells but only rarely
caused syncytium formation. This effect with RSV was most prominent in cells infected on day 7 post-harvest. For both viruses a maximum of 20% of the cells were positive for viral antigen by immunofluorescent antibody staining even in the most receptive (i.e. older) cells. It was not possible to quantify the number of cells exactly since some fields revealed single positive cells and others had clusters of positive cells. With PIV3, syncytia as well as some individual cells were antigen-positive. By 48 h after infection with PIV3 there was marked destruction of the cell layer and giant cell formation. In RSV-infected cells, not all the rounded cells were antigen-positive and syncytium formation was rare.

We have shown that both RSV and PIV3 can replicate and produce infectious virus in this model of monocytes/macrophages. The ability of a number of viruses to replicate and produce new infectious virus in peripheral blood monocyte/macrophages has been previously demonstrated (Arbeit et al., 1982; Daniels et al., 1978; Domurat et al., 1985; Sullivan et al., 1975; Van der Logt et al., 1980). Previous studies have demonstrated the presence of RSV antigens in peripheral monocytes/macrophages in vitro and in vivo (Domurat et al., 1985; Roberts, 1982) but did not determine whether infectious progeny resulted. New virus production in cultured bovine alveolar macrophages after infection with bovine RSV and bovine PIV3 has been previously demonstrated (Toth & Hesse, 1983). C.p.e. with syncytium formation after bovine parainfluenza virus, but not RSV, infection was also noted.

Ageing of monocytes in culture has been associated with maturation to more macrophage-like cells (Fischer et al., 1976). This ageing has been shown to allow increased replication of herpes simplex virus (HSV) (Daniels et al., 1978), varicella zoster virus (Arbeit et al., 1982), rubella (Van der Logt et al., 1980) and lentiviruses (Narayan et al., 1983), whereas measles virus replication is not increased under these conditions (Sullivan et al., 1975). Both alveolar macrophages and circulating monocytes are likely to be exposed to RSV and PIV3 during lower respiratory tract infections; however, the relationship of ageing in vitro to differentiation and maturation of monocytes to macrophages in vivo is unresolved.

Even under the most permissive conditions less than 20% of the monocytes infected with either virus were positive for antigen by immunofluorescent antibody staining. The reasons for this are unknown. Possible explanations for this heterogeneity include differences in cell cycle at time of infection, differences in cell differentiation, separate subpopulations of monocytes, or transient modulation by environmental factors (Morahan et al., 1985). Frequently, especially with RSV-infected cells, clusters of positive cells were noted in some areas, suggesting a role for local factors and cell-to-cell spread requiring cell contact.

In an effort to explain the restricted replication of RSV and PIV3 in the younger monocytes, we measured the production of HuIFN-α by RSV- or PIV3-infected cells in vitro (Fig. 2). After
infection with PIV3, the youngest cells produced the highest titres of HuIFN-α whereas older cells produced progressively less HuIFN-α. HuIFN-α production correlated inversely with the amount of viable PIV3 detected in the supernatants.

In contrast, the monocytes/macrophages infected with RSV produced progressively more HuIFN-α with longer ageing in vitro before exposure to the virus. However, RSV production paralleled that of PIV3 and was lowest in the cells infected after 1 day in tissue culture. After infection with either virus, maximal HuIFN-α production was detected 24 h after exposure to the virus. HuIFN-α was not detected in the 2 h specimens (collected immediately after viral adsorption) or in any of the supernatants from mock-infected cells (data not shown). The observed HuIFN-α activity was completely blocked when anti-HuIFN-α antibody was added to the VSV plaque-reduction assay system (data not shown).

In this study PIV3 production in monocyte/macrophage cultures correlated inversely with HuIFN-α production and directly with ageing of monocytes in vitro. Whether the HuIFN-α produced in response to the PIV3 in these cells is important in limiting the amount of virus production in the younger cells is not certain at present. A similar relationship has been noted in monocytes/macrophages infected with HSV in an analogous manner (Linnavuori & Hovi, 1982). With RSV infection the same trend of increasing viral titre in older cells was noted, but the youngest cells produced minimal amounts of HuIFN-α as determined in the standard bioassay. These findings parallel clinical observations that RSV is a poor inducer of HuIFN-α in the human host whereas PIV3 is not (Hall et al., 1978, 1981; Roberts, 1982). This suggests also that other factors in fresh monocytes may restrict RSV replication. For the two viruses it is possible that different monocyte/macrophage subsets are infected and this is responsible for the observed differences in HuIFN-α production. Another possible explanation for our findings is that the VSV plaque-reduction assay is not detecting RSV-induced HuIFN-α because of a difference in the antiviral spectrum of such HuIFN-α as suggested by Bell et al. (1983). It may be that the larger amounts of RSV antigen produced by older cells are capable of stimulating production of more HuIFN-α.

In conclusion, we have demonstrated that RSV and PIV3 induce productive infection in adherent human peripheral blood mononuclear leukocytes in vitro, and for both agents new virus production is increased in older, more macrophage-like cells. For PIV3, the amount of virus produced correlated inversely with the production of HuIFN-α by the cells, whereas with RSV infection the correlation was direct. Further investigations of the subsets of cells infected and the
role of HuIFN-α in response to each agent may help elucidate the role of monocytes/macrophages and HuIFN-α in the natural course of infection with RSV and PIV3.

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