Degradation of herpes simplex virions by human polymorphonuclear leukocytes and monocytes

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The degradation of herpes simplex virus particles after uptake by phagocytes was studied, but, since lysis of the phagocyte also resulted in damage to the viral envelope, measurement of viral infectivity as a criterion of viral degradation after phagocytosis was not possible. Therefore we focused on later events in viral destruction, namely the degradation of macromolecules. We have demonstrated that polymorphonuclear leukocytes (PMN) and monocytes (MN) can rapidly degrade the membrane proteins of the phagocytosed herpesvirus virions. PMN and MN from a patient with chronic granulomatous disease showed a similar rate of degradation compared to PMN and MN from healthy donors, which excludes an important role for toxic oxygen species in viral protein degradation. Experiments using toxic oxygen species-generating systems supported this observation. In contrast to PMN, MN are also effective in the digestion of viral DNA. We conclude that PMN and MN are able to neutralize large amounts of phagocytosed HSV, so their role in antiviral defence has again been demonstrated.

As well as specific defence mechanisms, such as T cells, it has recently become clear that phagocytes also contribute to antiviral defence. For herpes simplex virus (HSV) the role of phagocytosis by polymorphonuclear leukocytes (PMN), at least in keratitis, seems to be established (Smith et al., 1986; Bingham et al., 1985). We (Van Strijp et al., 1989a,b) and others (Bingham et al., 1985; Fujisawa et al., 1987; McCullough et al., 1988; Smith et al., 1986; West et al., 1987) have shown that viruses, including HSV, are efficiently phagocytosed by PMN and monocytes (MN). However, the importance of phagocytosis in antiviral defence can not be limited to the phenomenon of uptake alone. The ultimate question remains: can a phagocyte really destroy the virion in such a way that it is no longer infectious? The efficiency of this process will determine whether the phagocyte itself is at risk of becoming infected, or whether the phagocyte is a reservoir of live virus particles that are liberated and infect new targets elsewhere in the body, after the phagocyte has died. In the latter case the phagocyte could protect virus particles against other defence mechanisms and instead of being helpful to the host, the phagocyte may contribute to the capacity of the virus to invade target tissues. It could also be that the complex microbicidal activities of the phagocyte are virucidal in vivo and in vitro, in which case the phagocyte could be a key cell in the host defence against virus infections. MN can be infected by viruses and the probability of being infected is greater when specific anti-virus antibodies are present. This antibody-enhanced infection has been described for several viruses, such as Sindbis virus (Chanas et al., 1982), dengue virus (Halstead, 1988), lactate dehydrogenase-elevating virus (Inada & Mims, 1985), rabies virus (King et al., 1984), West Nile virus (Peiris et al., 1981), human immuno-deficiency virus (Takeda et al., 1988), BK virus (Traavik et al., 1988), yellow fever virus (Schlesinger & Brandriss, 1981) and fish rhabdoviruses (Clerx et al., 1978).

The ability of phagocytes to digest their bacterial targets has been the subject of several investigations (Babior, 1984; Spitznagel, 1984). Because we are interested in the role of the phagocytic cell in the host defence against HSV, we studied the question of whether phagocytes could degrade HSV. The classic method of studying inactivation of virions is neutralization of infectivity, measured by titration of the virions in plaque assays or TCID_{50} experiments. Unfortunately, after the virion is taken up by a phagocyte it is impossible to develop a method for lysing the cell and to free the intact virions in order to assay them in virus titrations, because the viral membrane is also damaged by the procedure. Furthermore, virions are already partially neutralized by the opsonins needed for phagocytosis. Therefore, we directly measured the capacity of the phagocyte to...
degrade viral protein and DNA. For the study of protein degradation we took advantage of the properties of Triton X-114 (Tx-114), a non-ionic detergent that can displace and replace much of the normal lipid environment around hydrophobic proteins, whereas hydrophilic proteins bind almost no detergent. This results in detergent micelles containing the integral membrane proteins and these micelles form aggregates at a temperature above 20 °C, resulting in a phase separation. After centrifugation they separate into a detergent-rich pellet containing hydrophobic proteins and a supernatant containing the soluble proteins and peptides (Bordier, 1981; Pryde, 1986).

PMN, MN, cell lines, viruses and human sera were obtained as described (Van Strijp et al., 1989a, b). PMN were 98% pure and 97% viable, and MN were highly enriched (80 to 90% purity). In our earlier work (Van Strijp et al., 1989a, b) it was shown by flow cytometric analysis that viruses did not associate with the contaminating lymphocytes and that phagocytes react normally in, for instance, uptake of bacteria and metabolic burst. No difference was observed between phagocytes of donors that were seropositive or seronegative for HSV. Virus was radiolabelled using 20 μCi [3H]thymidine per ml or methionine-free medium supplemented with 35 μCi [35S]methionine per ml, and purified as described (Van Strijp et al., 1989a). Virus was stored at −70 °C in small volumes at a standard concentration of 350 μg/ml. Purity was checked by electron microscopy and it was shown that over 95% of the particles were enveloped viruses. To determine phagocytosis of the virus PMN or MN were mixed with purified fluorescein-labelled virus and human serum, incubated for various time periods and measured on a per cell basis in a FACStar flow cytometer as described (Van Strijp et al., 1989a). In the absence of opsonins no uptake by PMN of HSV particles was observed, but spontaneous uptake of virions by MN reached a level of 25% after a 2 h incubation period. Addition of opsonins to this assay enhanced the uptake of virions for the phagocytes, but for MN the uptake process proceeded over a longer time (Van Strijp et al., 1989a). On the basis of the above mentioned measurements, for the following experiments, in which the degradation of HSV glycoproteins was studied, we used a 30 min phagocytosis time with radiolabelled virus. [35S]Methionine was used to label the viral proteins. A total of 60 μl PMN or MN [3 × 10⁷ cells/ml Hanks’ balanced salt solution with 5% foetal calf serum (HBSSf)] were mixed with 30 μl purified 35S-labelled virus and 30 μl human serum (diluted in HBSSf). The cells were incubated for 30 min in a shaking water-bath at 37 °C (150 r.p.m.) and the reaction was stopped by adding ice-cold phosphate-buffered saline. Cells were separated from free virus by centrifugation at 160 g and washed three times. The percentage uptake in 30 min, relative to the added amount of radioactivity, was 61 ± 3% for PMN and 52 ± 4% for MN, for the [35S]methionine as well as for the [3H]thymidine-labelled virions (about 8000 c.p.m. per 2 × 10⁶ cells, depending on the virus batch).

Thereafter the cells were washed, incubated further and then analysed for virus degradation. Cells were resuspended in 120 μl HBSSf and incubated further for various time periods at 37 °C in a shaking water-bath. After incubation the reaction mixtures were cooled on ice, solubilized in 1% Tx-114 in 500 μl and layered onto 500 μl 6% (w/v) sucrose. Tx-114 micelles were allowed to form aggregates at 37 °C and were then pelleted through the sucrose at 12000 g for 3 min. The Tx pellet and two 200 μl samples from the top supernatant were mixed with 2-5 ml scintillation cocktail and counted. Results were expressed as the solubilizable protein fraction: c.p.m. in supernatant fluid/(c.p.m. in pellet + supernatant fluid) × 100%.

In an initial experiment using only purified [35S]methionine-labelled virions it was determined that the virion-associated 35S label recovered in the detergent-rich phase remained the same after incubating the virion at 37 °C up to 2 h, demonstrating the validity of the choice for this system of using [35S]methionine as a label (data not shown). The data in Fig. 1 (percentage of degradation after uptake) are expressed as the percentage of radioactivity that was associated with Tx-114 relative to the amount that was taken up by the leukocyte. The results indicated that degradation of virion proteins took place both in MN and granulocytes and was time-dependent. In MN degradation was more effective than in granulocytes and reached values of over 70%, following an incubation period of 120 min after 30 min.
Table 1. Comparison of degradation rate of viral proteins by PMN from CGD patient and control cells

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<tr>
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<th>Control PMN</th>
<th>CGD PMN</th>
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<tr>
<td>Uptake (%)*</td>
<td>63.1 ± 2.6</td>
<td>61.7 ± 3.5</td>
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<tr>
<td>0 min†</td>
<td>21.1 ± 0.6</td>
<td>22.0 ± 0.7</td>
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<tr>
<td>60 min</td>
<td>39.1 ± 1.1</td>
<td>39.1 ± 0.7</td>
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<tr>
<td>120 min</td>
<td>48.1 ± 1.3</td>
<td>45.3 ± 1.4</td>
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* Percentage uptake expressed as the PMN-associated radioactivity as a percentage of total added radioactivity.
† Time after phagocytosis. Tx-114 non-associated percentage of total phagocytosed radioactivity.

The absence of a role for toxic oxygen species in viral protein degradation was further indicated by experiments carried out with PMN of a patient with chronic granulomatous disease (CGD). The results presented in Table 1 demonstrate that the phagocytic capacity of CGD PMN was the same as for control PMN and that even without the production of toxic oxygen species degradation of proteins occurred.

To study the degradation of viral DNA, PMN or MN were incubated with [3H]thymidine-labelled virions in the presence of opsonins. Non-ingested virions were washed away and the cells were incubated further at 37 °C. Thereafter cells were resuspended in water, frozen and thawed, then an equal volume of 10% TCA was added and incubated for 30 min at 4 °C. The precipitate
formed was centrifuged for 10 min at 12000 g and washed with 5% TCA. The pellet was suspended in Aqualuma, counted in a liquid scintillation counter and the TCA-precipitable radioactivity was measured. As expected, no breakdown of DNA was observed in PMN (Fig. 3). However, in MN after 30 min of incubation substantial DNA damage had already been observed. Two h later one-third of the phagocytosed DNA appeared to be broken into fragments that were no longer precipitable in 5% TCA, and thus they were smaller than about 20 bases (Cleaver & Boyer, 1972).

Phagocytosis of viruses results, when IgG is present, in a metabolic burst of the phagocyte (Bingham et al., 1985; Van Strijp et al., 1989b; Weber & Peterhans, 1983). The question still remains as to whether the viruses are actually neutralized or even degraded by the phagocytes. There are several reasons to assume that degradation of virions occurs. After phagocytosis and phagosome-lysosome fusion (Van Strijp et al., 1989a) it is likely that virus particles are inactivated and eventually degraded, due to the antiviral effects of lysosome constituents, such as defensins, cationic proteins (Rouse et al., 1980; Selsted & Harwig, 1987; Thorne et al., 1984; Zerial et al., 1987; Daher et al., 1986) and the reactive oxygen species liberated by the PMN. It has been shown that toxic oxygen products are able to inactivate virus infectivity (Belding et al., 1970). Initially we monitored the loss of infectivity of herpes virions after phagocytosis by PMN and MN according to the method that we developed to follow the degradation of *Escherichia coli* (Rozenberg-Arksa et al., 1984). After phagocytosis the phagocyte was lysed to release the virions, which were then quantified by titration on fibroblasts. The methods we used for disruption of the phagocyte (freezing and thawing, hypotonic shock, detergent lysis and sonication) destroyed the cells as well as the virions and resulted in non-reproducible titration curves of the released virions. Disruption using a Dounce homogenizer, a commonly used method for the lysis of virus-infected cells, unfortunately failed to work for these small amounts of phagocytes. To solve these technical problems we concentrated on a later stage of destruction of virions, namely the degradation of viral macromolecules, keeping in mind that when viral DNA or viral proteins are degraded the virus will no longer be infectious. Because one or more of the membrane proteins of HSV are responsible for the initiation of cell infection (Johnson et al., 1984) the degradation of membrane proteins can be viewed as a measure of loss of infectivity. In this study we showed that only MN were able to degrade the viral DNA to fragments that were no longer acid-precipitable. This indicated that the complete virion must have been fragmented beforehand, because enveloped virions are insensitive to DNase activity. In accordance with other findings for these cells, PMN were not capable of degrading viral DNA because they do not possess DNase (Lamers et al., 1981; Rozenberg-Arksa et al., 1984).

For the study of protein degradation we used Tx-114, which results in detergent micelles containing the integral membrane proteins that can be pelleted at temperatures above 20 °C (Bordier, 1981; Pryde, 1986). We showed a time-dependent decrease in the ability of radiolabelled proteins to integrate in Tx-114 micelles, mediated both by PMN and MN, and conclude that virions are broken down by these phagocytes and that MN perform more efficiently than PMN. But MN can become infected by several viruses and antibody-enhanced infection has been demonstrated in a number of cases. PMN escape infection, probably because of their differentiated state in which no DNA replication, RNA or protein synthesis can be observed (Bainton et al., 1971; Cline, 1966; Jack & Fearon, 1988).

Although we were able to show that viruses are degraded in phagocytes, it is not possible to determine the contribution of oxygen radicals to damage. PMN from a patient with CGD showed the same rate of degradation and cell-free oxygen radical-generating systems were inactive, indicating a minor role for oxygen radicals in the degradation of proteins. Furthermore, from the 10000 virus particles able to be taken up by phagocytes (Van Strijp et al., 1989a) some might escape the destruction machinery of the phagocyte. We propose that PMN are the best candidates for the clearance of virions and although monocytes show faster degradation patterns, the total number of PMN present and the
inability to be infected by viruses renders PMN more effective in vivo.

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References


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