Key words: HSV-1/immune response/killer T cells

Mechanism of Recognition of Herpes Simplex Virus Type 1-infected Cells by Natural Killer Cells

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SUMMARY

Human fibroblast FS-4 cells when infected with herpes simplex virus type 1 (HSV-1) become susceptible to lysis by purified populations of T3− human natural killer (NK) lymphocytes. Blocking of HSV-1 protein synthesis or N-linked glycosylation with pactamycin or tunicamycin, respectively, prevented HSV-1-infected cells from being lysed, suggesting that HSV-1 glycoprotein synthesis is required for recognition by NK cells. However, pactamycin- and tunicamycin-treated cells expressed on their membranes a detectable amount (20 to 40% of the untreated control) of HSV-1 glycoproteins gB, gC and gD, left by the virus during its internalization. Phosphonoformic acid (PFA) blocked HSV-1 DNA replication and inhibited the synthesis and surface expression of newly made gC, gD and gB by 90, 80 and 60% respectively. Despite this reduction, PFA treatment had no effect on NK susceptibility. The target structure recognized seems to be different from those expressed on tumour target cells since there was no competition for the lysis of HSV-1-infected FS-4 by K-562 or HeLa tumour target cells. However, a monoclonal antibody specific for the human transferrin receptor which inhibited NK recognition of tumour cells also blocked NK cytotoxicity of HSV-1-infected cells. In summary our results indicate that although viral glycoprotein synthesis is required, gB, gC and/or gD alone are not the targets for NK recognition of HSV-1-infected cells. In addition, they suggest the involvement of the host cell transferrin receptor in the NK killing process.

INTRODUCTION

Natural killer (NK) cells were initially described as capable of spontaneous, non-major histocompatibility-restricted lysis of a variety of tumour cells and certain normal cells (Kiessling & Wigzell, 1981; Ortaldo & Herberman, 1984). The effector cells responsible for this cytotoxicity comprise a small proportion of human peripheral blood lymphocytes (PBL), which belong to a special subpopulation of lymphoid cells with surface markers associated with the T cell and macrophage lineage (Lanier & Phillips, 1986). NK cells are unique among lymphocytes because they lack immunological memory and can kill without prior sensitization (Ortaldo & Herberman, 1984).

NK cells become activated (Biron & Welsh, 1982) and proliferate (Biron et al., 1983) during the early stages of virus infection and it has been hypothesized that they play a role in natural resistance to virus infections (Welsh, 1978). Many reports indicate that HSV-1-infected cells (Ching & Lopez, 1979; Yakusawa & Zarling, 1983; Liebson et al., 1986) are more sensitive to NK lysis in vitro than uninfected cells but this is not a general rule (Muñoz et al., 1983). Increased sensitivity may result from the induction of interferon (IFN) during the assay (Casali et al., 1981), although no correlation between levels of IFN and NK activity has been found.

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susceptibility to HSV-1 in mice (Lopez et al., 1982; Munoz et al., 1983; Bishop et al., 1983b). However, the role of NK cells in resistance to HSV-1 infections in vivo is still controversial (Welsh, 1981; Bukowski & Welsh, 1986; Rager-Zisman et al., 1987). Correlations between genetically high NK cell activity and resistance to HSV-1 have been noted and depletion of NK cells by various means enhances susceptibility to HSV-1 in mice (Lopez et al., 1980; Habu et al., 1984).

In recent years, Bishop et al. (1983a, 1984, 1986) have studied this system of NK cytotoxicity against HSV-1-infected cells as a model to study NK recognition. They have found that cells infected with HSV-1 mutants defective in expression of some gC and gD glycoproteins have reduced susceptibility to lysis and that monoclonal antibodies (MAbs) against gB or gC partially blocked NK lysis.

In the present study we have used metabolic inhibitors of HSV-1 replication as tools to modify the level of expression of the major glycoproteins on the surface of the infected cells. We have found that expression of early but not late gene products seems to be a requisite for susceptibility to NK lysis and that no simple correlation exists between the levels of gB, gC and gD expression and susceptibility to NK lysis by T3- effector cells. Furthermore, MAbs against the human transferrin receptor blocked NK lysis of HSV-1-infected cells. Our results suggest that virus-induced over-expression or modification of the transferrin receptor could cause recognition by NK cells.

METHODS

Materials. Dulbecco's modified Eagle's medium (DMEM) and sera were obtained from Gibco. Ficoll-Hypaque and Percoll were purchased from Pharmacia. Human lymphoblastoid interferon (HuIFN-α: 1-6 × 10⁴ international units (IU)/ml protein) was a generous gift from Drs N. B. Finter, K. H. Fantes & M. D. Johnston, Wellcome Research Laboratories, Beckenham, U.K.). Sodium [¹¹⁵C]chromate (250 to 500 mCi/ml) and other radioactive chemicals were purchased from Amersham. Tunicamycin, pactamycin and phosphoformic acid (PFA) were a generous gift of Dr Luis Carrasco (Universidad Autonoma). MAbs raised against T3 and the human transferrin receptor were kindly supplied by Dr F. Sánchez-Madrid (Universidad Autonoma, Madrid) and human antiserum against HSV-1 glycoproteins, by Dr E. Tabarés (Universidad Autonoma, Madrid).

Cells and viruses. The epithelial cell line HeLa, derived from a cervical carcinoma, and the human fibroblast cell line FS-4 were cultured in DMEM supplemented with 10% newborn calf serum (NCS) or 10% foetal calf serum (FCS), respectively. The NK-sensitive target K-562 is a human erythroleukaemia cell line from a patient with chronic myelogenous leukaemia and was cultured in DMEM containing 10% FCS and antibiotics. All lines were mycoplasma-free when used as target cells.

HSV-1 (KOS strain) was grown on HeLa cells in DMEM supplemented with 2% NCS. Twenty-four h after infection, cells were collected and the intracellular virus was extracted by successive freezing and thawing cycles followed by low speed centrifugation. The supernatant was used as the source of virus.

Effector cells. Heparinized venous blood was obtained from normal, HSV-1 seronegative, adult volunteers. Purified PBL were separated by standard Ficoll-Hypaque density centrifugation. Cells were pelleted, washed three times and resuspended in DMEM/2% FCS. In most experiments purified NK populations were obtained. Briefly, after depletion of cells adhering to plastic flasks, non-adherent cells were separated by centrifugation on discontinuous Percoll density gradients (37, 40, 43, 46 and 53%). NK activity was found in the 40% Percoll layer that contains mainly large granular lymphocytes (LGL) (Alarcón & Fresno, 1985). LGL were sometimes depleted of T cells by treatment with OK-T3 MAb plus complement. The resulting population was less than 2% T3+ and less than 4% monocytes as assessed by immunofluorescence.

Cytotoxicity assay. NK cytotoxicity was evaluated with a ⁵¹Cr release assay. HSV-1-infected or mock-infected FS-4 cells were used as target cells. NK assay was carried out as described (Munoz et al., 1983). Briefly, cells were seeded at 10⁴ cells/well in microtitre plates and infected or mock-infected with HSV-1 at an m.o.i. of 10 p.f.u./cell. After 60 min incubation at 37 °C the non-adsorbed virus was removed and fresh DMEM with 2% FCS was added. At different times the medium was removed and the cells were incubated for 30 min with 50 μl DMEM containing 50 μCi/ml sodium [¹¹⁵C]chromate. Target cells were then washed twice and the effector cells added at different effector to target (E:T) ratios in 100 μl of DMEM plus 2% FCS. Cultures were incubated 4 h at 37 °C. Spontaneous release of ⁵¹Cr by target cells was determined in the absence of effector cells and was less than 25% in all cases. Supernatants (50 μl) were collected and counted in an LKB automatic gamma counter. One-hundred per cent release was determined by adding 100 μl 0·1 M-NaOH containing 1% SDS to the adherent cells.

The cytotoxic activity was also measured in lytic units. One lytic unit (LU) is arbitrarily defined as the number of cells that gave 30% specific release of ¹⁰⁶ target cells. These numbers were determined from graphs plotting specific release against the number of effector cells as estimated from regression analysis of results using six different E:T ratios from 100:1 to 5:1.
NK recognition of HSV-1-infected cells

Cold Target Inhibition Assay. Cold target inhibition assay was carried out by adding unlabelled cells at a 5:1 excess to cultures used for the 51Cr release assay. HSV-1-infected or mock-infected FS-4 cells were used as target cells. NK assay was carried out as described above.

Immunoprecipitation. Cells were iodinated using Iodogen (Pierce Chemicals, Rockford, Ill., U.S.A.) (Fraker & Speck, 1978) and cell lysates were prepared with 10 mM-Tris-HCl pH 8-0, 140 mM-NaCl, 1% Triton X-100, 1% haemoglobin and 1.5 mM-PMSF. The lysates (400 µl) from 107 cells were mixed with 75 µl antisera supernatants for 2 h at 4°C. To isolate immune complexes, 30 µl of the rat 187.1 anti-mouse kappa chain MAb coupled to Sepharose beads (1 ng/ml) (gift from Dr T. Springer, Harvard University, Boston, Mass., U.S.A.) was added. After shaking for 2 h, the beads were washed twice with phosphate-buffered saline (PBS) containing 0.1% Triton X-100 and 0.1% haemoglobin, twice with PBS and once more with 30 mM-Tris-HCl pH 6-6. Immunoprecipitates were subjected to SDS-PAGE and autoradiography (Nabel et al., 1981).

Treatment of Infected Cells with Metabolic Inhibitors. FS-4 cells were pretreated for 30 min at 37°C with pactamycin (10⁻⁷ M) at the indicated times after infection, then washed three times with PBS. Cells were also treated with tunicamycin (10 µg/ml) or PFA (15 or 30 µg/ml) from the moment of HSV-1 addition. Pactamycin and tunicamycin were washed off before 51Cr labelling. PFA was maintained in the medium for the entire NK assay. In some NK experiments the infected cells were treated with 10⁻⁷ M-pactamycin for 30 min at 37°C at the end of the infection period before 51Cr labelling. Treatment with metabolic inhibitors did not significantly affect spontaneous 51Cr release.

Protein Synthesis. Protein or glycoprotein synthesis was evaluated by the incorporation of the radioactive precursors [³⁵S]methionine, [³H]mannose or [³H]glucosamine respectively. HSV-1-infected FS-4 cells were washed once either in methionine-free or glucose-free DMEM and incubated for 2 h at 37°C with the same medium containing 5 µCi/ml of [³⁵S]methionine (1000 Ci/mmOl or 10 µCi/ml of D-[6-³H]glucosamine (35 Ci/mmOl plus 10 µCi/ml of [³H]mannose (50 Ci/mmOl respectively. After incubation the cells were washed with PBS and resuspended in 0.1 ml sample buffer (62.5 mM-Tris-HCl pH 6-8, 1% SDS, 0.1 mM-dithiothreitol, 17% glycerol) and analysed by SDS-PAGE as described (Nabel et al., 1981).

RESULTS

HSV-1-infected fibroblasts were the targets of NK cells

To study NK recognition of HSV-1-infected cells, we used FS-4 cells which were resistant to lysis by purified T3+ NK cells. When FS-4 cells were infected with HSV-1 and used as targets at different times after infection, a time-dependent susceptibility to lysis by NK cells was observed, reaching a plateau at 8 h post-infection (p.i.) (Fig. 1). In contrast, infected cells pretreated with pactamycin (10⁻⁷ M), an irreversible inhibitor of protein synthesis, to block the synthesis of new viral proteins were not lysed. Similarly, cells grown in the presence of tunicamycin (10 µg/ml), to inhibit N-linked glycosylation, were not lysed. Addition of pactamycin 4, 8 or 12 h p.i. did not inhibit NK lysis and in fact increased the lytic susceptibility slightly (Fig. 1).

HSV-1-infected cells were killed because of having target structures different from those of K-562 or HeLa cells since a fivefold excess of these unlabelled cells could not inhibit lysis of the infected FS-4 cells, in agreement with previous reports (Fitzgerald et al., 1983; Muñoz et al., 1983). By contrast, HSV-1-infected HeLa cells could inhibit lysis to the same extent as infected FS-4 cells (Fig. 2). Addition of free virus was ineffective (not shown).

Characterization of the Target Structure

The above results suggested that some glycosylated HSV-1-induced structure present at early times after infection was responsible for the susceptibility to lysis of infected FS-4 cells. The most obvious candidates for this role were the major glycoproteins gB, gC and gD as has been suggested by Bishop et al. (1983 a, 1984, 1986). Therefore, we further investigated the presence and synthesis of these three proteins in FS-4-infected cells. As it had been previously shown that HSV-1 internalization by host cells leaves some HSV-1 glycoproteins in the host cell membrane (Peake et al., 1982), we tested the amount of HSV-1 present in the cell membranes by external labelling and subsequent immunoprecipitation with an antisera from a seropositive patient. As shown in Fig. 3 the three major glycoproteins of HSV-1 were detected in the cells 4 h p.i. Interestingly, the cells pretreated with pactamycin before infection with HSV-1 (used at the same m.o.i. as in Fig. 2), which were resistant to NK lysis, also had a detectable amount of gB,
Fig. 1. NK lysis of HSV-1-infected FS-4 cells. HSV-1-infected FS-4 cells (10^4/well) were labelled with ^{51}Cr and used as target cells in a standard 4 h release assay using PBLs (△). Cells pretreated with pactamycin (10^{-7} M) for 30 min before infection (□). Cells treated with pactamycin (10^{-7} M) for 30 min at the indicated times after infection (○). Cells cultured in presence of tunicamycin (10 µg/ml) for the entire period of time before the NK assay (●). Results shown are the means of triplicate cultures.

Fig. 2. Competition for lysis of HSV-infected FS-4 cells. HSV-1-infected FS-4 cells, treated for 30 min with pactamycin 8 h p.i. and then labelled with 51 Cr were used as target cells as in Fig. 1. No competitor cells added (□), or a 5:1 excess of unlabelled K-562 (■), HeLa (▲), HSV-1-infected HeLa 8 h p.i. (△), or HSV-1-infected FS-4 cells 4 h p.i. (●).

Table 1. Effect of metabolic inhibitors on glycoprotein synthesis in HSV-1-infected FS-4 cells

<table>
<thead>
<tr>
<th>Inhibitor added</th>
<th>[^{35}S]Methionine</th>
<th>N-Acetyl[^{3}H]glucosamine</th>
<th>^{3}H]Mannose</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>155250 (100)</td>
<td>6871 (100)</td>
<td>4947 (100)</td>
</tr>
<tr>
<td>Tunicamycin</td>
<td>119970 (78)</td>
<td>2215 (32)</td>
<td>289 (6)</td>
</tr>
<tr>
<td>PFA</td>
<td>157843 (102)</td>
<td>2031 (29)</td>
<td>1243 (25)</td>
</tr>
<tr>
<td>Pactamycin</td>
<td>1384 (1)</td>
<td>195 (3)</td>
<td>104 (2)</td>
</tr>
</tbody>
</table>

* Cells were incubated with radioactive precursor for 1 h at 8 h p.i. and radioactivity insoluble in TCA was determined as described in Methods.

† FS-4 cells were pretreated for 30 min with pactamycin before HSV-1 infection or treated with tunicamycin or PFA from the beginning of the infection.

gC and gD on their membranes. Similar results were observed with tunicamycin-treated cells (not shown). Densitometric scanning of the gels shown in Fig. 3 indicated that the level of expression of pactamycin-treated HSV-1-infected cells was about 20 to 40% of the control cells, depending on the experiment (not shown).

Since expression of the major glycoproteins on the cell surface did not correlate with NK susceptibility, we tested whether de novo synthesis showed a better correlation. For this purpose we used the metabolic inhibitors of virus replication pactamycin, tunicamycin and PFA, an inhibitor of viral DNA synthesis (Perrin & Stünzi, 1984). As shown in Table 1, pactamycin pretreatment completely blocked protein synthesis in FS-4 cells 8 h p.i. In our system 8 h p.i., infected FS-4 cells synthesized mainly HSV-1-induced proteins due to the shut-off of host cell protein synthesis (Muñoz & Carrasco, 1984). As expected, tunicamycin mainly inhibited the incorporation of radioactive sugar precursors (N-acetylglucosamine and mannose) into proteins and PFA had no effect on overall protein synthesis although the level of glycosylation was
reduced. The proteins synthesized under the above conditions were analysed by SDS-PAGE (Fig. 4). Tunicamycin blocks the incorporation of oligosaccharides into mature glycoproteins, although the precursor molecules pC and pD were detectable. PFA, which inhibits DNA replication and therefore the production of the late (γ) proteins (Perrin & Stünzi, 1984; Wagner, 1985), drastically reduced the levels of gC (and concomitantly of pC), the synthesis of which is dependent on viral DNA replication (Spear, 1985), by more than 90%. Furthermore, the highest concentration (30 μg/ml) of PFA also inhibited the synthesis of gB and gD, defined as early (β) protein (Wagner, 1985), by an average of 60 and 80% respectively. Similar inhibitions of the amount of internally labelled gB, gC and gD expressed on cell membranes were observed (results not shown).

However, despite the reduction in expression of the three major glycoproteins, HSV-1-infected FS-4 cells were equally susceptible to lysis by purified human NK cells (Fig. 5). The summary of all of the above experiments comparing gB, gC and gD synthesis and/or expression

Fig. 3. Immunoprecipitation analysis of 125I-labelled HSV-1-infected FS-4 cells. The cells were labelled externally with 125I and immunoprecipitated with a serum from a seropositive donor and analysed by SDS-PAGE. Lane 1, control HSV-1-infected FS-4 cells 4 h p.i.; lane 2, the same but pretreated with pactamycin (10^-7 M) for 30 min before infection.
on the cell membrane, as well as susceptibility to NK lysis is shown in Table 2. No clear correlation between gB, gC and gD expression and/or their synthesis and NK susceptibility was apparent.

We have shown previously that the transferrin receptor could be one of the target structures recognized by NK cells (Alarcón & Fresno, 1985) and that some MAbs against it blocked NK recognition (J. A. López-Guerrero et al., unpublished data). Therefore, we tested the effect of these MAbs on NK lysis of HSV-1-infected cells. MAb FG 2/12 blocked NK lysis of HSV-1-infected cells, whereas other MAbs directed at different epitopes of the transferrin receptor were ineffective. Interestingly, MAb FG 1/5 selectively inhibited NK lysis of HeLa cells (Table 3).

Fig. 4. SDS-PAGE analysis of the HSV-1 glycoproteins synthesized in the presence of metabolic inhibitors. The cells were labelled for 2 h with a mixture of N-acetyl[3H]glucosamine and [3H]mannose. Uninfected FS-4 cells (A), HSV-1-infected FS-4 cells 8 h p.i. (B), treated with 1, 5 or 30 μg/ml PFA (C, D and E respectively) or 10 μg/ml tunicamycin (F) from the beginning of infection. Densitometric scans of the corresponding lanes are shown at the left.
Fig. 5. NK activity against HSV-1-infected FS-4 cells treated with different metabolic inhibitors. Samples of the cells used in Fig. 4 treated with tunicamycin (1) or PFA (1) (30 μg/ml) or both (1) or neither (1) were used as target cells. They were additionally treated for 30 min with pactamycin to prevent further protein synthesis. Untreated T3- cells (a) or (b) boosted with IFN-α (400 IU/ml) for 16 h before the assay were used as effector cells.

Table 2. Correlation of HSV-1 glycoprotein synthesis and surface expression with NK susceptibility

<table>
<thead>
<tr>
<th>Inhibitor added*</th>
<th>Glycoprotein (%)†</th>
<th>Glycoprotein (%)§</th>
<th>Glycoprotein (%)¶</th>
<th>Glycoprotein (%)∥</th>
<th>Glycoprotein (%)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gB</td>
<td>gC</td>
<td>gD</td>
<td>gB</td>
<td>gC</td>
</tr>
<tr>
<td>Tunicamycin (10 μg/ml)</td>
<td>4</td>
<td>50</td>
<td>4</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>Pactamycin (10–7 M)</td>
<td>4</td>
<td>30</td>
<td>2</td>
<td>40</td>
<td>3</td>
</tr>
<tr>
<td>PFA (30 μg/ml)</td>
<td>40</td>
<td>ND§</td>
<td>8</td>
<td>ND</td>
<td>20</td>
</tr>
</tbody>
</table>

* Cells were pretreated with pactamycin before HSV-1 infection or treated with PFA or tunicamycin from the beginning of infection.
† Results of SDS–PAGE densitometric analysis of proteins synthesized (S) or expressed in the membrane (M) 8 h p.i. of inhibitor-treated cells. The values are given as the percentage of synthesis/expression of the control cells.
‡ Percentage of control LU of purified T3- NK cells using infected FS-4 cells as targets.
§ ND, Not determined.

DISCUSSION

Our results show that HSV-1-infected fibroblasts are susceptible to lysis by purified human NK cells. However, in contrast to most of the other systems used (Bishop et al., 1983a; Fitzgerald et al., 1983; Colmenares & Lopez, 1986), uninfected FS-4 cells were basically resistant to lysis by NK cells, and therefore represent a good system to study NK recognition of HSV-1-infected cells.

Bishop et al. (1983a, 1984, 1986) have done extensive work on the characterization of the target structure(s) recognized by NK in HSV-1-infected cells, and conclude that NK cells recognize the three major glycoproteins (gB, gC and gD) and that this recognition is clonally distributed. However, our results indicated that the mere expression of these three proteins on the surface of the infected cell is not sufficient to render the FS-4 cell susceptible to NK lysis.
Table 3. Effect of MAbs specific for the transferrin receptor on NK lysis of HSV-1-infected cells

<table>
<thead>
<tr>
<th>MAb*</th>
<th>HSV-1-infected 50:1</th>
<th>HSV-1-infected 50:1</th>
<th>HSV-1-infected 20:1</th>
<th>HSV-1-infected 20:1</th>
<th>HeLa 50:1</th>
<th>HeLa 50:1</th>
<th>HeLa 20:1</th>
<th>HeLa 20:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>60</td>
<td>43</td>
<td>66</td>
<td>38</td>
<td>37</td>
<td>28</td>
<td>42</td>
<td>30</td>
</tr>
<tr>
<td>FG 1/5</td>
<td>54</td>
<td>52</td>
<td>20†</td>
<td>9†</td>
<td>36</td>
<td>30</td>
<td>25†</td>
<td>15†</td>
</tr>
<tr>
<td>FG 1/6</td>
<td>73</td>
<td>45</td>
<td>68</td>
<td>42</td>
<td>56</td>
<td>37</td>
<td>44</td>
<td>29</td>
</tr>
<tr>
<td>FG 2/12</td>
<td>21†</td>
<td>14†</td>
<td>30†</td>
<td>18†</td>
<td>21†</td>
<td>18†</td>
<td>21†</td>
<td>13†</td>
</tr>
<tr>
<td>FM 2/2</td>
<td>66 ND†</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>29 ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Purified MAbs (20 μg/ml) were added to HSV-1-infected FS-4 cells 10 h p.i. or to HeLa cells before purified T3- NK cells, and the cytotoxicity assay was carried out as described.
† Significantly different from the controls, P < 0.001.
‡ ND, Not determined.

since the internalization of the virus leaves some gB, gC and gD on the membrane, even in the presence of protein synthesis inhibitors such as pactamycin or cycloheximide (Peake et al., 1982). The amount of HSV-1 glycoproteins left in FS-4 cell membranes in the presence of pactamycin is approx. 20 to 40% of the proteins expressed in control infected cells when optimal lysis was observed. However, there was no lysis by NK cells. This result suggests that HSV-1 replication is needed for NK recognition. A similar conclusion was reached by Bishop et al. (1983a) who showed that the protein synthesis inhibitor emetine inhibited lysis by NK cells. In contrast, PFA treatment significantly reduced gB, gC and gD expression but did not prevent NK lysis. Therefore, we could not demonstrate a clear correlation between gB, gC and gD expression and NK susceptibility.

Our studies with tunicamycin suggest that glycosylation is required for NK susceptibility. In the presence of tunicamycin, HSV-1 precursor polypeptides were transported and expressed in host cell membranes (Norrild & Pedersen, 1982; Spear, 1985), but lysis was abolished. Bishop et al. (1983a) have shown that another glycosylation inhibitor, 2-deoxy-D-glucose (2-dG), only partially reduced (30 to 40%) NK lysis. Although both compounds inhibit the formation of N-linked oligosaccharides, the mechanism of action is not exactly the same (Schwarz & Datema, 1982). As the same group showed, 2-DG inhibited the formation of the three mature glycoproteins gB, gC and gD, although the precursor forms of gC and gD were expressed on cell membranes whereas the precursor of gB was not (Glorioso et al., 1983). Immature gC and gD allowed the complement-mediated lysis of HSV-1-infected cells by specific antibody against them. Based on this, the authors claimed that 2-DG only affects NK recognition of gB. However, the fact that antibodies still recognize precursor forms of gC and gD does not necessarily imply that the same is true for NK recognition.

The following hypothesis could explain our results and those of Bishop et al. (1983a, 1984, 1986). If NK cytotoxicity plays a role in resistance to HSV-1, it is likely that recognition is directed against an early component of the infected cells that is encoded or induced by the virus, before the release of newly formed infective virus occurs. NK cells may recognize a host protein which, when expressed on cell membranes, is enhanced indirectly as a consequence of the infection or directly, because it becomes associated with HSV-1 glycoproteins. It is well known that HSV-1 glycoproteins follow a transport route before being inserted into the host membrane. During this transport glycosylation occurs and HSV-1 glycoproteins may become associated with this putative target protein. Glycosylation inhibitors that block this transport may therefore block NK recognition. This hypothesis explains why the inhibitory effects of several gC- and gB-specific MAbs were not additive (Bishop et al., 1984). These authors were unable to conclude from their experiments whether NK cells recognize limited regions of the viral proteins, and suggest the possibility that the antibodies exerted their effects by sterically interfering with access to determinants not encoded by HSV-1. On the other hand, a single
amino acid change in gB in a mar mutant of HSV-1 was sufficient to decrease the clonal frequency more than twice (Bishop et al., 1986). These results may imply that this residue is the target for more than half of the NK population, which is surprising especially if recognition of HSV-1 by NK cells is clonally distributed as it is in T cells. Alternatively, the mutation may alter the association with the putative target protein. On the other hand, although the syn mutant used by Bishop et al. (1983a) is defective in gC expression, it is also known from other studies that the altered expression of some HSV-1 glycoproteins may affect the expression of others (Norrild & Pedersen, 1982; Spear, 1985).

In agreement with our hypothesis, Colmenares & López (1986) have shown that tumour target cells susceptible to lysis by NK or natural cytotoxic (NC) effector cells were more susceptible to lysis when they were infected by HSV-1. However, the effector cells did not show cross-reactivity. NC effector cells did not kill HSV-1-infected NK target cells and vice versa, suggesting that host cell determinants also play a role in NK recognition. Using another herpesvirus, human cytomegalovirus (CMV), Borysiewicz et al. (1985) reported that PFA did not affect the susceptibility of infected cells to NK lysis and the authors suggested that the target structure was host cell-derived and enhanced coincidentally with CMV early antigen expression. Furthermore, the same group reported evidence that the susceptibility of CMV-infected cells to NK lysis correlated with transferrin receptor expression by the host cells (Borysiewicz et al., 1986). This molecule has been proposed to be one of the target molecules recognized by NK cells in tumour target cells (Vodinelich et al., 1983; Alarcón & Fresno, 1985) and MAbs against it, such as FG 2/12, also blocked NK lysis of tumour cells. Our results with MAbs suggest that the transferrin receptor is one of the host cell proteins recognized by NK cells, and that its expression is enhanced or altered by the synthesis and/or transport of HSV-1 glycoproteins. It is apparent that this hypothesis is difficult to reconcile with the lack of cold target competition by tumour target cells (Fig. 2) (Muñoz et al., 1983). However, this discrepancy may be explained by proposing that the structure of the transferrin receptor is in some way altered during its association with HSV-1 glycoproteins since MAb FG 1/5 which blocks NK activity against tumour cells did not block it against HSV-1-infected cells.

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