Processing of human cytomegalovirus glycoprotein B in recombinant adenovirus-infected cells

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Intracellular processing of human cytomegalovirus (HCMV) glycoprotein B (gB; gpUL55) expressed by a recombinant adenovirus (Ad-gB) was studied in human A549 cells as processing events could affect immunogenicity when such viruses are used as live-recombinant vaccines. Cleavage of [35S]methionine-labelled gp130 into gp93 and gp55 reached a maximum after a 3 h chase. Cleavage was completely inhibited by brefeldin A, suggesting that processing normally occurs as a late Golgi or post-Golgi event. Uncleaved gp130 remained completely sensitive to endo-p-N-acetylglucosaminidase H (Endo-H) in untreated cells following long chase periods, indicating high-mannose oligosaccharides at all of the 18 N-linked glycosylation sites (Asn-X-Ser/Thr) and retention in the endoplasmic reticulum.

Endo-H analysis of gp55 from swainsonine-treated and untreated cells was consistent with glycosylation at all three potential sites, with two oligosaccharides remaining sensitive to Endo-H and one being processed to Endo-H resistance. The heavily glycosylated N-terminal gp93 subunit was not detected by [35S]methionine-labelling but was easily detected along with gp55 after labelling with [3H]mannose. No cleavage of gp130 was observed in analogous pulse-chase radiolabelling of Ad-gB-infected human fibroblasts, even though these cells are permissive for HCMV replication and can process the native gB molecule. Processing of gB in recombinant adenovirus-infected A549 cells was generally similar to that previously reported for native gB in HCMV-infected fibroblasts.

Introduction

Human cytomegalovirus (HCMV) is the most common microbial agent causing birth handicap and is a major cause of life-threatening illness in immunocompromised individuals (Alford & Britt, 1993). Although the protective efficacy of a live-attenuated HCMV vaccine has been demonstrated (Plotkin et al., 1991), theoretical concerns about latency and potential oncogenicity of live herpesvirus vaccines have prompted researchers to develop inert HCMV subunit vaccines or live-recombinant viruses expressing HCMV immunogens (Marshall & Plotkin, 1993).

Glycoprotein B (gB; gpUL55) is a major component of the HCMV envelope that bears sequence and structural homology to gB of herpes simplex virus type 1 (HSV-1) (Cranage et al., 1986; Spaete et al., 1988). This glycoprotein has been previously identified as the most promising candidate for inclusion in HCMV subunit vaccines (Marshall & Plotkin, 1993). gB is the predominant target of neutralizing antibodies during natural HCMV infection (Britt et al., 1990; Gönczöl et al., 1991; Rasmussen et al., 1991; Marshall et al., 1992, 1994), and inoculations of humans with native gB produce neutralizing antibodies and cellular responses (Gönczöl et al., 1990).

The gB gene of HCMV encodes a primary translational product of 906 (AD169 strain) or 907 (Towne strain) amino acids with typical features of type I viral membrane glycoproteins (Cranage et al., 1986; Spaete et al., 1988): a cleaved N-terminal signal peptide (amino acids 1-24), a large ectodomain with multiple sites for N-linked oligosaccharides [Asn-X-Ser/Thr where X is not proline (Kornfeld & Kornfeld, 1985)], and a hydrophobic transmembrane domain (amino acids 715–748 for Towne strain). Co-translational glycosylation, signal peptide cleavage, protein oligomerization and folding occur in the endoplasmic reticulum (ER) of HCMV-infected cells prior to transport of the gB precursor gp130 [referred to as gp150 by some authors (Britt & Vugler, 1989)] to the Golgi apparatus (Britt & Vugler, 1989, 1992).

Transport through the Golgi is necessary for oligosaccharide maturation and proteolytic cleavage of gp130 [between amino acids 460 and 461 for Towne strain (Spaete et al., 1988, 1990)] into the mature N-terminal gp93 subunit [referred to as gp116 by some authors (Britt & Vugler, 1989)] and the membrane-anchored gp55 subunit [referred to as gp58
Fig. 1. Processing of [35S]methionine-labelled gB in Ad-gB-infected A549 cells. Radioimmunoprecipitation of lysates from pulse-chase (0–8 h) labelled cells was performed using C23 monoclonal antibody, and precipitated proteins were treated (+) or not treated (−) with Endo-H prior to SDS-PAGE. ‘Mock-infected’ refers to immunoprecipitated protein from lysates of pulse-labelled but uninfected A549 cells. Molecular mass markers (MRK) are shown on the left in this figure and in Figs 2-4. 

by some authors (Britt & Vugler, 1989)]. These exist as a disulphide-linked complex in extracellular virions (Britt & Vugler, 1989, 1992). Proteolytic processing of gp130 has been reported for cells infected with recombinant vaccinia virus expressing the gB gene (Cranage et al., 1986; Britt et al., 1990; Gönczől et al., 1991), as well as for truncated forms of the gB gene expressed transiently in COS cells or stably in Chinese hamster ovary (CHO) cell lines (Spaete et al., 1988, 1990).

The immunodominant neutralizing domain of gB is well conserved (Chou, 1992; Darlington et al., 1991) and is mapped to a continuous sequence in gp55 (amino acids 553–636 for Towne strain; Qadri et al., 1992; Wagner et al., 1992). A conserved linear neutralization epitope has also been mapped near the N terminus of gp93 and uncleaved gp130 (amino acids 68–84 in Towne strain), but not all HCMV-seropositive human sera react with this epitope (Meyer et al., 1992; Ohlin et al., 1993; Ayata et al., 1994). The gp93 subunit is heavily glycosylated (15 of 18 potential N-linked sites) compared to homologous gB proteins from murine CMV (nine sites; Rapp et al., 1992), and human herpesvirus-6 (five to eight sites; Chou & Marousek, 1992).

Among the recombinant vectors being considered for delivery of viral glycoprotein immunogens to humans, adenoviruses hold particular promise because of their stability, ease of manipulation, growth to high titre, and high level expression of heterologous genes (Berkner, 1988). Further, the potential for intranasal immunization with a recombinant adenovirus to induce mucosal immunity has been demonstrated (Gallichan et al., 1993). Live, enterically administered adenovirus vaccines have been used with demonstrated safety and efficacy for a number of years in the United States military (Rubin & Rorke, 1994). The construction and preliminary characterization of a recombinant adenovirus expressing HCMV gB (Ad-gB) was reported previously (Marshall et al., 1990, 1992). This recombinant virus expressed immunoreactive gB gene products and induced neutralizing antibodies in hamsters after intranasal inoculation. However, processing of cloned glycoproteins may differ from that in native virus-infected cells for a variety of reasons, including the absence of other proteins encoded by the native virus (Hutchinson et al., 1992), the presence of proteases or other functions encoded by the recombinant virus, or the cell type used for expression (Qadri et al., 1992). Structural features resulting from co- and post-translational processing events such as glycosylation, cleavage, disulphide bond formation and oligomerization can affect functional characteristics such as intracellular disposition, conformation and, ultimately, antigenicity (Machamer et al., 1985; Gething et al., 1986).
Methods

**Cells and viruses.** As previously reported (Marshall et al., 1990), the gene encoding Towne strain gB was cloned into the genome of adenovirus type 5 (Ad-5) downstream from the E3 promoter, deleting nonessential E3 sequences between map units 78.5 and 84.0. Ad-gB and the parental Ad-5 strain were propagated and studied in A549 cells (human lung carcinoma; ATCC) grown in Eagle’s minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS). Infected cells were lysed in conditioned medium by freeze-thawing three times and clarified supernatants were used as the inoculum in all experiments. Viral titres were established by counting infectious foci using indirect immunofluorescence 48 h post-infection. For immunofluorescence and \[^3S\]methionine labelling of infected A549 cells, an m.o.i. of 1 was used in order to mimic the physiological conditions expected during in vivo mucosal infection. For the \[^3H\]mannose labelling studies of infected A549 and human fibroblast cells (MRHF cells, Whittaker Bioproducts) the m.o.i. was approximately 0.1.

**Antisera.** The following antisera were used in these experiments. A polyclonal, monospecific guinea-pig antiserum raised to physically purified gB (González et al., 1986) was obtained from E. González, Wistar Institute, Philadelphia, PA. C23, a human monoclonal antibody against the linear neutralization epitope near the N terminus of gp130 and gp93 (Tomiyama et al., 1990; Meyer et al., 1992) was obtained from Y.-I. Matsumoto, Teijin Institute, Japan. Murine monoclonal 27-156, which recognizes an epitope in the immunodominant domain on gp55 (Wagner et al., 1992), was obtained from W. Britt, University of Alabama, Birmingham, Ala., USA.

**Metabolic labelling and pharmacological treatments.** Pulse-chase radiolabelling was initiated 24 h post-infection. Protein labelling of infected and uninfected cells was accomplished by pulse-chasing for 30 min with \[^35S\]methionine (35 or 110 \(\mu\)Ci/ml TRAN35S-label; ICN Biomedicals) in methionine-free EMEM, followed by 8-h chase intervals utilizing EMEM containing 0.1 mM nonradioactive methionine and 2% FBS. Glycoprotein labelling was accomplished by pulse-chasing for 30 min with \[^3H\]mannose (200 \(\mu\)Ci/ml; ICN Biomedicals) in glucose-free EMEM, followed by chase intervals with EMEM containing 2.0 mM nonradioactive mannose. In some experiments, brefeldin A (Epicentre Technologies) was added to medium at 2.5 \(\mu\)g/ml or swainsonine (Genzyme) at 2.0 \(\mu\)g/ml, each concurrent with pulse-labelling and continuing through the chase intervals.

**Radioimmunoprecipitation.** Cells were harvested, washed, pelleted and lysed by sonication in buffer containing 150 mM-NaCl, 20 mM-Tris (pH 7.5), 1% NP40, 1% deoxycholate and 0.1 mM-PMSF (Marshall et al., 1992). Lysates were clarified by ultracentrifugation and stored at −70 °C. Fifty \(\mu\)l of clarified cell lysate and 50 \(\mu\)l of antibody diluted in PBS with 1% Triton X-100 were incubated at 4 °C overnight. Protein-G agarose beads (Genex) were added for 1 h, collected by centrifugation, and washed five times with cold PBS containing 1% Triton X-100 and washed five times with cold PBS containing 1% Triton X-100. Bound antigen–antibody complexes were eluted from the beads by heating at 90 °C for 10 min in 100 mM-Tris (pH 8.0), 2% SDS, 40% glycerol, 0.1% Triton X-100 and 10% 2-mercaptoethanol. This supernatant was combined with an equal volume of 0.1% bromophenol blue and the protein G-agarose beads and then incubated overnight at 37 °C in the presence or absence of 2 \(\mu\)g/ml endo-\(\beta\)-N-acetylglucosaminidase H (Endo-H) (DuPont NEN) in buffer containing 0.1 M-sodium citrate (pH 5.5) and 1 mM-PMSF, prior to SDS-PAGE.

**Results**

**Glycosylation and post-translational processing of the gp130 precursor protein**

Infected cells demonstrated maximal gB-specific immunofluorescence 24–36 h post-infection, exhibiting a cytoplasmic staining pattern (data not shown). Pulse-labelling of Ad-gB-infected cells with radioactive precursors (amino acids or monosaccharides) was therefore initiated 24 h post-infection, followed by chase intervals up to 8 h in duration. Co- and post-translational processing of gB gene products was analysed by pulse-chase radiolabelling with \[^35S\]methionine and radioimmunoprecipitation of cell lysates with gB-specific antibody. In addition, immunoprecipitates were subjected to Endo-H digestion in order to discriminate between high-mannose, precursor type oligosaccharides (Endo-H sensitive) and Golgi-processed, complex oligosaccharides (Endo-H resistant; Kornfeld & Kornfeld, 1985). As shown in Fig. 1, the glycosylated precursor gp130 was the major product at the end of the 30 min pulse-labelling, and radiolabel in gp130 persisted at high levels throughout the 1–8 h chase intervals. The large reduction in size of gp130 after Endo-H digestion (approximately 35–40 kDa) was consistent with high-mannose oligosaccharides at most or all of the 18 potential N-linked glycosylation sites. Radiolabelled protein with an approximate molecular mass of 260 kDa also maintained Endo-H sensitivity throughout the pulse-labelling, consistent with a dimeric form of gp130 that persisted during electrophoresis despite the presence of SDS and reducing agent. The retention of complete Endo-H sensitivity for gp130 monomers and dimers during extended chase intervals suggested that most of the newly synthesized gp130 never exited the ER.

Radiolabel at the top of the polyacrylamide slab gel (Fig. 1) was also reduced in size by Endo-H digestion and may have represented higher oligomers of gp130. An Endo-H-sensitive, 29 kDa glycoprotein present at the bottom of the gel may have represented a degradation product of gB previously detected by immunoblotting of Ad-gB-infected cell lysates (Marshall et al., 1990). Nonspecifically precipitated polypeptides (non-glycosylated, based upon unchanged mobility after Endo-H digestion) included the 200 kDa and 45 kDa polypeptides from mock-infected cells and major adeno viral polypeptides (100, 70 and 60 kDa) from Ad-gB-infected cells.

Although a small amount of gp55 was present at the end of the pulse-labelling along with uncleaved gp130, a major increase in this C-terminal cleavage product was observed between the 1 and 2 h chase intervals. Radiolabelled gp55 persisted through the 8 h chase interval. The expected N-terminal cleavage product, gp93, was not detected above background radioactivity in the immunoprecipitates, even
though gp93 and gp55 should have been equivalently labelled with [35S]methionine [eight methionines for gp93 and nine methionines for gp55 (Spaete et al., 1988)].

Endo-H digestion reduced the size of gp55 by approximately 4 kDa and generated a second minor species approximately 6 kDa smaller than gp55, suggesting the removal of two oligosaccharides from the major species and five oligosaccharides from the minor species. Partial resistance of the major gp55 species to Endo-H indicated processing in the medial-Golgi, where exoglycosidases and glycosyltransferases convert high-mannose and hybrid oligosaccharides (Endo-H sensitive) to complex oligosaccharides (Endo-H resistant; Kornfeld & Kornfeld, 1985). The same pattern of Endo-H digestion products for gp55 was observed even after longer chase intervals, indicating that this heterogeneity was due to incomplete processing rather than slow processing at one of the N-linked glycosylation sites.

Because gp93 should be much more heavily glycosylated than gp55 [15 versus 3 N-linked glycosylation sites (Spaete et al., 1988)], [3H]mannose was substituted for [35S]methionine in pulse-labelling of Ad-gB-infected A549 cells. As shown in Fig. 2 (left panel), both gp93 and gp55 were easily detected by immunoprecipitation following a chase interval of 1 h or more, with gp93 exhibiting a relatively heterogeneous size distribution. In addition, [3H]mannose labelling eliminated much of the non-specific background due to adenoviral polypeptides (Fig. 1). C23, the monoclonal antibody to the N-terminal gp93/gp130 epitope, was more efficient in precipitating [3H]mannose-labelled gp55 than 27-156, a monoclonal antibody to a gp55 epitope, or polyclonal antiserum to gB (data not shown). This indicated that gp55 must have been complexed with gp93 (or uncleaved gp130) at the time of the immunoprecipitation. No radiolabelled gB gene products were detected in immunoprecipitates of extracellular medium from pulse-chase labelled cells (data not shown), indicating that gp93 was not secreted.

With analogous [3H]mannose labelling of human fibroblasts infected with the same low m.o.i. used for A549 cells, less radiolabel was incorporated into gB and no proteolytic processing of gp130 was detected (Fig. 2, right panel). This result was unexpected because human fibroblasts are permissive for HCMV and produce virions with proteolytically cleaved gB (Britt & Vugler, 1989). The previous detection of gp55 by immunoblot in lysates of MRC human fibroblasts infected with Ad-gB at the high m.o.i. of 50 (Marshall et al., 1990) suggests that transport and processing are concentration-dependent.

**Proteolytic processing of gp130 is blocked by brefeldin A**

To better localize the intracellular site where proteolytic cleavage of gp130 occurs, Ad-gB-infected A549 cells were treated with brefeldin A, which inhibits ER to Golgi transport by redistributing cis- and medial-Golgi enzymes into the ER
Fig. 3. Effect of brefeldin A on proteolytic cleavage of gp130. Ad-gB-infected A549 cells were pulse-labelled with [35S]methionine then chased for 0-4 h. Radioimmunoprecipitation of cell lysates was performed using polyclonal antiserum to gB, and precipitated proteins were treated (+) or not treated (−) with Endo-H prior to SDS-PAGE.

Endo-H...

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Swainsonine modifies oligosaccharide processing but not proteolytic cleavage

The N-linked oligosaccharide content of gp55 was further investigated by treating Ad-gB-infected A549 cells with swainsonine during pulse-chase radiolabelling with [35S]methionine. Swainsonine specifically inhibits α-mannosidase II in the medial-Golgi (Kang & Elbein, 1983), resulting in the accumulation of hybrid oligosaccharides [(NeuNAc+Gal-GlcNAc-Man3GlcNAc2-Asn, Endo-H sensitive (Hunt & Wright, 1981)] in place of fully processed, complex oligosaccharides [(NeuNAc-Gal-GlcNAc)_2-Man3GlcNAc2-Asn, Endo-H resistant]. As expected for a precursor protein resident in the ER, the mobility of immunoprecipitated gp130 before and after Endo-H digestion was unaffected by swainsonine treatment of Ad-gB-infected cells (Fig. 4). Neither transport of gp130 from the ER to the Golgi nor proteolytic cleavage were affected by swainsonine. However, the major gp55 species after Endo-H digestion (2 and 4 h chase samples in Fig. 4) was approximately 2 kDa smaller for swainsonine-treated versus untreated cells, and this ‘deglycosylated’ gp55 (retaining only the proximal N-acetylglucosamine linked to asparagine at each of the three sites) was equivalent in size to the minor species detected after Endo-H digestion in Fig. 1. These results further supported the conclusion that all three available N-linked glycosylation sites on gp55 were utilized, but only one of these oligosaccharides was usually processed to a complex, Endo-H-resistant structure during transit through the Golgi.

Another expectation of swainsonine treatment was the production of a population of gp93 with oligosaccharides remaining completely sensitive to Endo-H, so that size heterogeneity due to oligosaccharide heterogeneity (Fig. 2, left panel) would be eliminated. However, a new discrete protein species equivalent to ‘deglycosylated’ gp93 was not detected in Endo-H-digested protein from the 2 h and 4 h chase samples. Nevertheless, a more intense band was seen in the region of Endo-H-digested gp55 from swainsonine-treated cells (especially the 2 h chase sample in Fig. 4), suggesting that ‘deglycosylated’ gp93 and gp55 might have co-migrated during SDS-PAGE. The expected mass of these moieties would be similar (436 amino acids and 15 GlcNAc for gp93 versus 447 amino acids and 3 GlcNAc for gp55 (Spaete et al., 1988)).

Discussion

This study demonstrated that co- and post-translational processing of gB in recombinant adenovirus-infected A549 epithelial-like cells was similar to that previously described in HCMV-infected human fibroblasts (Britt & Vugler, 1989, 1992). The gB polypeptide was co-translationally modified in the ER by the addition of multiple N-linked, high-mannose oligosaccharides, and gp130 was then slowly and inefficiently transported to the Golgi, followed by oligosaccharide maturation and proteolytic cleavage to gp93 and gp55. In both HCMV-infected human fibroblasts (Britt & Vugler, 1989, 1992) and Ad-gB-infected A549 cells (Figs 1, 2 and 4), gp55 and gp93 accumulated to maximal amounts between 1 h and 3 h after pulse-labelling, but large amounts of unprocessed
gp130 were still retained in the ER during longer chase intervals.

The cellular origin of the endoprotease involved in cleavage of gp130 to gp93 and gp55 has been established in previous studies of recombinant gB expressed in vaccinia virus-infected CV-1 cells (Cranage et al., 1986; Vey et al., 1995) or HeLa cells (Britt et al., 1990), in transfected COS cells (Spaete et al., 1988), and in recombinant CHO (Spaete et al., 1988, 1990) or astrocytoma cells (Reis et al., 1993). Furin, a subtilisin-like endoprotease, has been identified as an HCMV gB-processing enzyme (Vey et al., 1995) with specificity for the Arg-X-Lys/Arg-Arg peptide motif. In the current study, inhibition of gp130 cleavage by brefeldin A in Ad-gB-infected A549 cells demonstrated that ER to Golgi transport was necessary for processing to occur. This has also been demonstrated for the gB homologue of pseudorabies virus in brefeldin A-treated swine cells (Whealy et al., 1991) and for native and recombinant HCMV gB in cells treated with the ionophores monensin (Britt & Vugler, 1989) or A23187 (Spaete et al., 1990). Although brefeldin A treatment allowed slow processing of oligosaccharides to Endo-H-resistant structures (Fig. 3; Whealy et al., 1991), no cleavage of the gB precursor was detected. This suggested that the cellular endoprotease was not redistributed in an active form to the ER, and was most likely localized to the trans-Golgi or trans-Golgi network (Griffiths & Simons, 1986).

One difference in processing of gB in recombinant adenovirus-infected A549 cells versus native gB in HCMV-infected fibroblasts was the apparent absence of uncleaved gB with Golgi-processed oligosaccharides in the former (Figs 1 and 2). A higher-molecular-mass form of gB with partial Endo-H resistance (mature ‘gp160–170’ versus precursor ‘gp150’) was detected in the cell-associated fraction and in extracellular virions of HCMV-infected human fibroblasts (Britt & Vugler, 1989), and this mature but uncleaved gB was also detected on the surface of recombinant vaccinia virus-infected HeLa cells (Britt et al., 1990).

A major question that arises from the present studies with adenovirus-expressed gB (Figs 1 and 4) and previous studies of native gB (Britt & Vugler, 1989, 1992) or recombinant vaccinia virus-expressed gB (Britt et al., 1990) concerns the inability to detect significant quantities of the N-terminal gp93 subunit by immunoprecipitation of [35S]methionine-labelled cells, even though this protein should be present in quantities similar to gp55 in a disulphide-linked gp93–gp55 complex (Britt &

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Fig. 4. Effect of swainsonine on oligosaccharide processing of gB. Ad-gB-infected A549 cells were pulse-labelled with [35S]methionine and chased for 0–4 h, in the presence (SW) or absence (UT; untreated) of swainsonine. Radioimmunoprecipitation of cell lysates was performed using polyclonal antiserum to gB, and precipitated proteins were treated (+) or not treated (−) with Endo-H prior to SDS-PAGE.
Because gp93 contains approximately five times as many N-linked oligosaccharides as gp55, radiolabelled gp93 could be visualized in quantities similar to gp55 using metabolic radiolabelling with \(^{3}H\)mannose (Fig. 2). Although gp93 may be less stable than gp55 and selectively degraded within or at the surface of cells, this explanation for the gp93 deficiency is not consistent with the detection of similar quantities of gp93 and gp55 at the surface of radio-iodinated extracellular virions (Britt & Vugler, 1989). HCMV-infected fibroblasts (Brett et al., 1990) and recombinant vaccinia virus-infected HeLa cells (Britt et al., 1990). This explanation is also inconsistent with the observation that cell-associated gp55 was immunoprecipitated just as efficiently with antibody specific for a gp93 epitope as with antibody of radio-iodinated extracellular virions (Britt & Vugler, 1989), HCMV-infected fibroblasts (Britt & Vugler, 1989, 1992). As with many other viral glycoproteins (Doms et al., 1993), oligomerization is necessary for exit from the ER and transport to the Golgi. Extensive post-oligomerization folding must also occur for HCMV gB prior to transport (Britt & Vugler, 1992), which might explain the long delay and inefficiency of gB processing. Another potential factor in ER retention of gB might be slow processing of the N-terminal signal peptide, as has been reported for human immunodeficiency virus type 1 gp160 (Li et al., 1994). A low and insufficient concentration of newly synthesized gB, resulting in insignificant amounts of oligomerized and properly folded gB, may explain why gp130 processing was not detected in Ad-gB-infected human fibroblasts (Fig. 4) and plasmid-transfected COS cells (Qadri et al., 1992). A block in ER to Golgi transport is a more likely explanation than the absence of the appropriate cellular endoprotease (Qadri et al., 1992), because appropriate cleavage occurred for native gB in HCMV-infected fibroblasts (Britt & Vugler, 1989) and for a recombinant, truncated gB in COS cells transfected with a high-level expression vector (Spaete et al., 1988).

For the fraction of gp130 in Ad-gB-infected A549 cells that was transported through the Golgi and cleaved into gp93 and gp55, processing of N-linked oligosaccharides was generally similar to that observed in HCMV-infected human fibroblasts in three respects. First, the heterogeneous size distribution for gp93 labelled metabolically with \(^{3}H\)mannose (Fig. 2), or extrinsically by radio-iodination (Britt & Vugler, 1989), was indicative of extensive heterogeneity in oligosaccharide structures at many of the N-glycosylation sites, similar to the diverse array of complex, hybrid and high-mannose oligosaccharides observed for the heavily glycosylated gp85 protein of avian retroviruses (Hunt et al., 1981). Second, the N-linked oligosaccharides of gp55 were incompletely processed in the Golgi, so that gp55 acquired only partial resistance to Endo-H digestion: an Endo-H-resistant complex oligosaccharide was present at one site and Endo-H-sensitive hybrid or high-mannose oligosaccharides at the other two sites (Figs 1 and 4). Similarly, only 3 of 8 kDa total carbohydrate was removed from gp55 by Endo-H in HCMV-infected cells (Britt & Vugler, 1989), suggesting two complex oligosaccharides and one hybrid or high-mannose oligosaccharide. Third, oligosaccharide processing was blocked with specific glycosidase inhibitors prior to acquisition of Endo-H resistance, without inhibiting ER to Golgi transport and proteolytic cleavage of gp130 (Fig. 4; Britt & Vugler, 1989).

The presence of less extensively processed, Endo-H-sensitive oligosaccharides at two of the N-glycosylation sites on gp55 is somewhat surprising, because the extent of processing is thought to reflect the relative physical accessibility of the oligosaccharides to exoglycosidases and glycosyltransferases as the native protein is traversing the Golgi apparatus (Kornfeld & Kornfeld, 1985; Hubbard, 1988). One of the gp55 N-glycosylation sites is only six amino acids downstream from the proteolytic cleavage site (Spaete et al., 1988), which is accessible to the cellular endoprotease, and the other two are within the immunodominant neutralizing domain of gp55 (Meyer et al., 1992; Ohlin et al., 1993), which is accessible to antibodies. One possibility is that the oligosaccharides within the immunodominant domain are "facing inward" in the oligomeric structure of gB and are therefore less accessible, as observed with one of the oligosaccharides of the mature trimeric HA glycoprotein from seal and fowl plague influenza viruses (Hunt et al., 1988).

Authentic co- and post-translational modification in the environment of recombinant adenovirus-infected human cells has been previously reported for cleaved viral glycoproteins such as HIV-1 gp160 (Dewar et al., 1989) and measles virus fusion protein (Alkhabit et al., 1990), as well as uncleaved viral glycoproteins such as HSV-1 gB (Johnson et al., 1988). The processing of HCMV gB in A549 cells infected with Ad-gB also appears to be authentic compared to gB processing in HCMV-infected cells, consistent with recognition of this recombinant gB by a variety of monoclonal and polyclonal antisera (Marshall et al., 1990, 1992, 1994). Therefore, in vitro-synthesized gB in individuals immunized with Ad-gB or analogous recombinant adenoviruses would be expected to express the same neutralizing epitopes as native gB.

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References


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