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Identification of the major immunogenic structural proteins of human foamy virus

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We have identified the major immunogenic structural proteins of the human foamy virus (HFV), a distinct member of the foamy virus subfamily of *Retroviridae*. Radiolabelled viral proteins were immunoprecipitated from HFV-infected cells by foamy virus antisera of human and non-human primate origin. Precipitated viral proteins were in the range of 31K to 170K. Labelling of proteins with [^{14}C]glucosamine or with [^{35}S]methionine in the presence of tunicamycin, as well as endo-β-*N*-acetylglycosaminidase H and F treatment of [^{35}S]methionine-labelled proteins, revealed three viral glycoproteins of approximately 170K, 130K and 47K, most likely representing the *env* gene-encoded precursor, the surface glycoprotein and the transmembrane protein of HFV, respectively.

Foamy viruses were first discovered in primary monkey kidney cell cultures in 1954, when the characteristic multinuclear giant cell c.p.e. with vacuolized 'foamy' cytoplasm appeared spontaneously after long-term cultivation (Enders & Peebles, 1954). Achong *et al.* (1971) isolated the first human foamy virus (HFV) from cultured cells of a nasopharyngeal carcinoma. Further reports on HFV infections are scarce. The isolation of virus from patients with subacute thyroiditis *de Quervain*, with toxic encephalopathy, and with chronic myeloid leukaemia has been described (Young *et al.*, 1973; Stancek & Gessnerova, 1974; Cameron *et al.*, 1978; Werner & Gelderblom, 1979); the diagnosis of foamy viral infection was confirmed by electron microscopy and immunofluorescence. Seroepidemiological surveys by indirect immunofluorescence assays revealed a seroprevalence of 3·4% and 6·9% among healthy donors from Uganda and the Pacific region, respectively (Loh *et al.*, 1980; Muller *et al.*, 1980). These results provide some evidence for a naturally occurring infection with HFV although an aetiological link to a specific disease is still missing (Teich, 1982).

The genome of HFV is similar to those of other human retroviruses (Maurer & Flügel, 1988). Besides the presumptive *gag*, *pol* and *env* genes, HFV contains at least four additional open reading frames. Three of these genes called *bel 1*, *2* and *3* are located between the *env* gene and the 3' long terminal repeat, whereas the fourth one, *S1*, is found between the *pol* and *env* genes (Flügel *et al.*, 1987; Maurer *et al.*, 1988). From the nucleotide sequence data (Flügel *et al.*, 1987; Rethwilm *et al.*, 1987; Maurer *et al.*, 1988), HFV *env*-encoded proteins of 102K, 54K and 47K in their unglycosylated forms would be predicted.

Wild-type HFV (a gift from Dr R. Flügel, Heidelberg, F.R.G.) was grown on BHK-21 cells or on human glioblastoma U-251 MG cells (Bigner *et al.*, 1981). Cultures were maintained in MEM supplemented with 5% foetal bovine serum, glutamine and antibiotics. Cells were infected with HFV at an m.o.i. of 0·1, and infection was monitored by assessment of the characteristic c.p.e. by light microscopy. Metabolic labelling, immunoprecipitation and SDS–PAGE were principally performed as described (Harlow & Lane, 1988). The final concentration of the radiochemicals (Amersham-Buchler) was 100 µCi/ml for [^{35}S]methionine, and 25 mCi/ml for [^{14}C]glucosamine. After labelling, the cells were lysed in 1 × RIPA (radioimmunoprecipitation analysis) detergent buffer (20 mM-Tris–HCl pH 7·4, 0·3 M-NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0·1% SDS, 1 mM-PMSF). Lysates were clarified by high-speed centrifugation and samples of 50 µl were reacted with 2·5 µl of serum. After incubation with Protein A–Sepharose beads (Pharmacia) and extensive washing with 1 × RIPA detergent buffer, antigens were eluted from immune complexes by boiling for 2 min and resolved by SDS–PAGE. After electrophoresis, the gels were fixed, soaked in fluorographic reagent (Amplify, Amersham), and then dried. Autoradiographic exposure to X-ray films (DuPont) was for 2 to 5 days at −70 °C.

Viral proteins from lysates of HFV-infected human glioblastoma U-251 MG and BHK-21 cells as well as

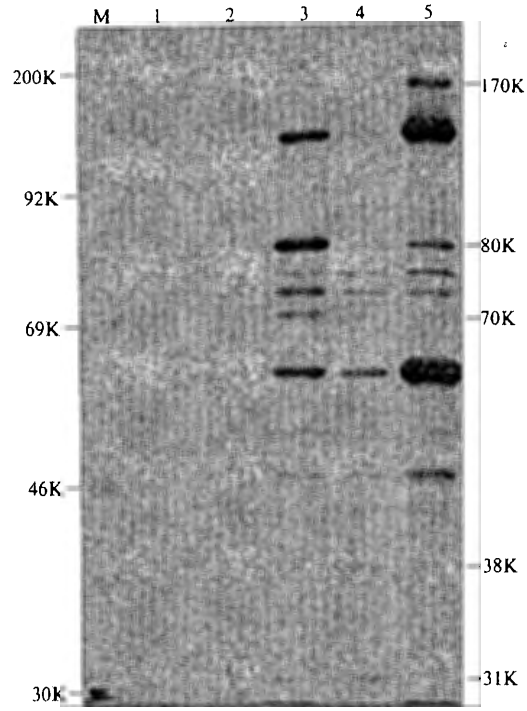

Fig. 1. SDS–PAGE analysis of immunoprecipitated HFV-specific proteins. Uninfected and HFV-infected BHK-21 cells were labelled with [^{35}S]methionine and then lysed as described. Lane 1, lysates of uninfected BHK-21 cells reacting with HFV-positive human serum C2; lane 2, lysates of HFV-infected cells reacting with foamy virus-negative human serum C1; lanes 3 to 5, lysates of HFV-infected cells immunoprecipitated with HFV-positive human sera C2, C3, and chimpanzee serum C4, respectively. Lane M, M_r markers.

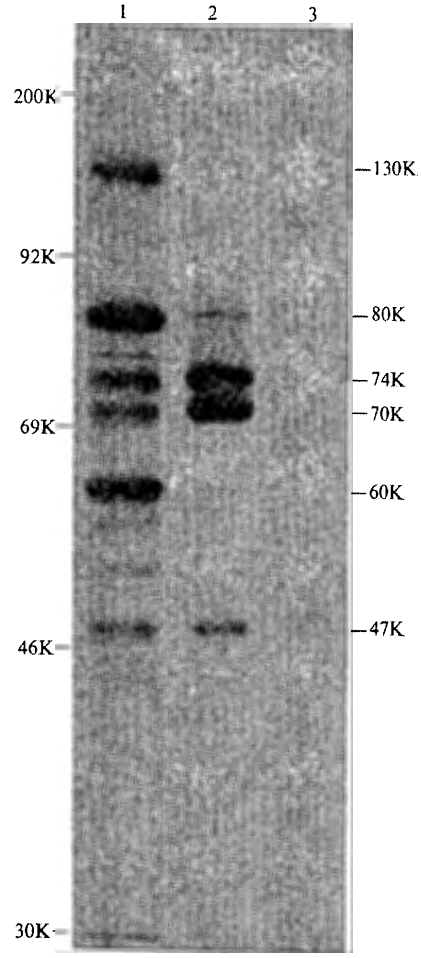

Fig. 2. Immunoprecipitation of HFV-specific proteins from lysates of [^{35}S]methionine-labelled pelleted virus. Lysates of HFV-infected BHK-21 cells were prepared (lane 1) and served as a control. From the corresponding culture supernatant, virus particles were pelleted by high-speed centrifugation and then lysed (lanes 2 and 3). Foamy virus-positive human serum C2 (lanes 1 and 2) and control serum C1 (lane 3) were then reacted with the lysates. The precipitated proteins were analysed by 7·5% SDS–PAGE. The sizes of the precipitated proteins are indicated.

from pelleted virus obtained from culture supernatant were immunoprecipitated with one human control serum antibody-negative for foamy virus (C1), and three sera (two human and one chimpanzee serum; C2, C3 and C4, respectively) which were shown previously to contain antibodies to foamy virus by an indirect immune fluorescence assay performed as described elsewhere (Neumann-Haefelin *et al.*, 1983). The three foamy virus-positive sera precipitated proteins of 170K, 80K, 70K, 38K and 31K specifically from lysates of HFV-infected cells (Fig. 1). Other proteins appeared in one or both of the control lanes. To determine which of these proteins were HFV-specific, we prepared lysates of [^{35}S]methionine-labelled pelleted virus particles. The proteins precipitable from these lysates were compared with those precipitable from extracts of infected cells (Fig. 2). It became evident that the proteins of 130K, 80K, 74K, 70K and 47K, and, to a small extent, also the 60K protein were part of HFV particles (Fig. 2, lane 2). The precipitated viral polypeptides were also found in infected U-251 MG cells, thereby indicating that there was no essential difference in HFV gene expression between cell lines of rodent and human origin (data not shown).

As for human lentiviruses, the largest HFV-specific proteins detected by immunoprecipitation are possibly *env* gene products and glycosylated. To test this assumption, we labelled infected BHK-21 cells with [^{14}C]glucosamine (Fig. 3*a*). Of the proteins usually precipitated from the [^{35}S]methionine-labelled lysates (lane 1), there was one readily identified as being glycosylated after short exposure times of the relevant

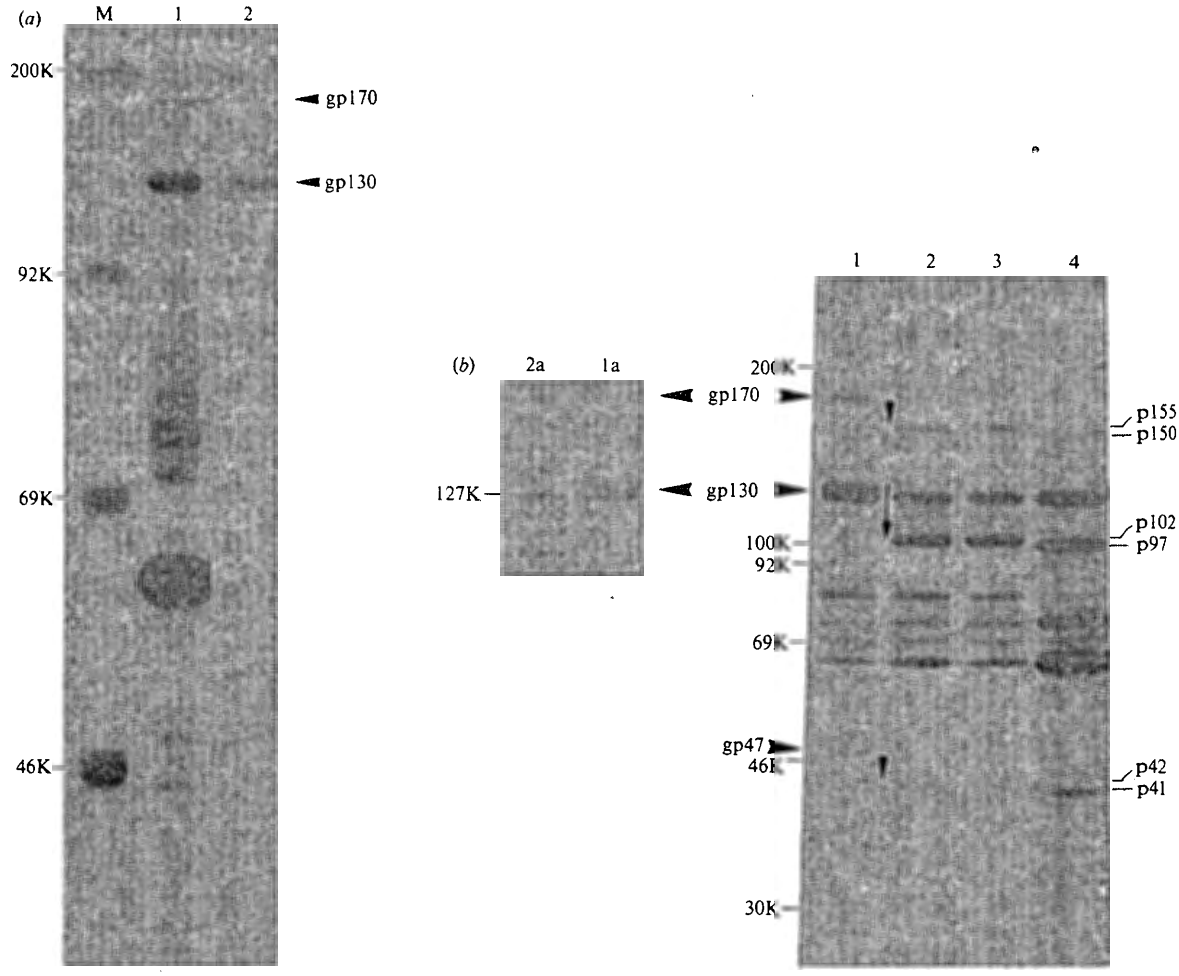

Fig. 3. Identification of HFV glycoproteins by [^{14}C]glucosamine labelling, endo-β-*N*-acetylglucosaminidase-F and -H and tunicamycin treatment. (*a*) HFV-infected BHK-21 cells were labelled with [^{35}S]methionine (lane 1), or with [^{14}C]glucosamine (lane 2). Cell lysates were then immunoprecipitated with HFV-positive chimpanzee serum C4, and resolved by 7·5% SDS–PAGE. The sizes of the HFV glycoproteins are indicated. (*b*) After labelling of HFV-infected BHK-21 cells in the absence (lanes 1 to 3, 1a and 2a) or presence of tunicamycin (lane 4), cell lysates where immunoprecipitated with the same HFV-positive serum. Proteins from normal lysates were incubated overnight at 37 °C either with buffer only (lanes 1 and 1a), with Endo-F (lanes 2 and 2a), or with Endo-H (lane 3). For comparison, proteins from tunicamycin lysates are shown (lane 4). Lanes 1a and 2a correspond to lanes 1 and 2, respectively, except that they were exposed at room temperature without an intensifying screen. gp170 is presumed to be the HFV *env*-encoded precursor; gp130 and gp47 are presumed to be the surface protein and transmembrane protein, respectively. The sizes of the corresponding deglycosylated and non-glycosylated proteins (prefixed 'p') are indicated at the right of the lanes.

[^{14}C]glucosamine labelling autoradiographs (lane 2), which was designated gp130. With longer exposure times (10 weeks), another band of approximately 170K became faintly visible. To verify the glycoprotein nature of these proteins, we treated immune-precipitated [^{35}S]methionine-labelled proteins with endo-β-*N*-acetylglucosaminidases F and H (Endo-F, Endo-H; Boehringer Mannheim) as described elsewhere (Lee *et al.*, 1984; Tarentino *et al.*, 1985). The incubation buffer contained 0·1 M-sodium citrate pH 5·5, 10 mM-EDTA, 0·1 M-2-mercaptoethanol, 0·1% SDS, 1 mM-PMSF and 1% Triton X-100. Cleavage of *N*-linked oligosaccharide side-chains of the high-mannose and complex types by Endo-F, and of the high-mannose type alone by Endo-H, showed a reduction in the apparent M_r of three proteins (Fig. 3*b*, lanes 2 and 3, respectively); gp170, gp130 and gp47 were reduced to 155K, 102K and 42K, respectively. There was no essential difference in the outcome of the two enzymic digestions which allows the conclusion that there is little if any glycosylation of the biantennary complex type.

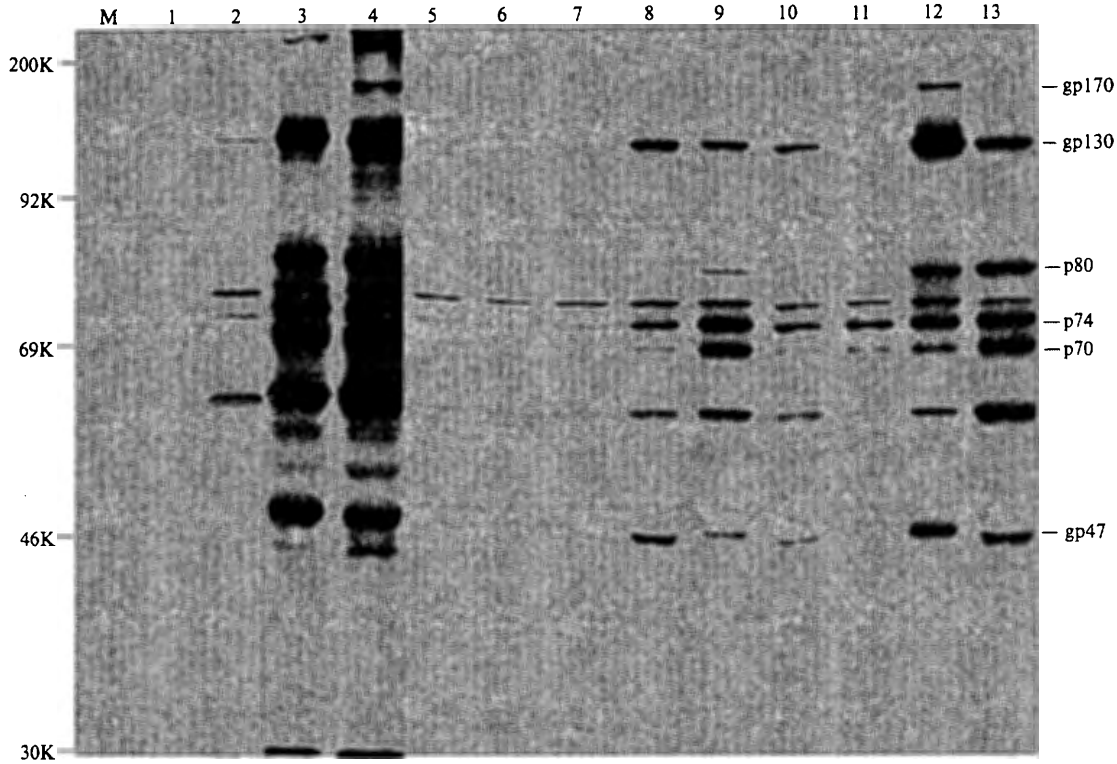

Fig. 4. Reactivity of monkey serum samples for antibodies to foamy virus as determined by RIPA/SDS–PAGE. Cell lysates were prepared from [^{35}S]methionine-labelled HFV-infected BHK-21 cells and immunoprecipitated with the following sera: lane 1, human control serum (T1) negative for foamy virus antibodies; lane 2, positive chimpanzee serum T2; lanes 3 and 4, two positive macaque sera (T3 and T4); lanes 5 to 7, three negative sera derived from macaques (T5 to T7); lanes 8 to 12, positive sera derived from macaques (T8 to T12); lane 13, a positive serum derived from an African green monkey (T13). The sizes of the recognized HFV proteins are indicated.

The protein band observed in the 130K range after Endo-F and -H digestions and after tunicamycin treatment (Fig. 3*b*, lanes 2 to 4 and 2a) represented a non-glycosylated HFV-specific protein of about 127K which could also be seen in the control lane (Fig. 3*b*, lane 1a) after shorter exposure times of the autoradiograph. Metabolic labelling with [^{35}S]methionine in the presence of 20 mg/ml tunicamycin prevented *N*-linked glycosylation (Fig. 3*b*, lane 4). Instead of the three glycoproteins gp170, gp130 and gp47, bands of approximately 150K, 97K and 41K appeared which represent the respective non-*N*-glycosylated protein cores. Since Endo-F and -H cleave carbohydrate side-chains between the first and second *N*-acetylglucosamine residue, the slightly higher M_r of the Endo-F and -H deglycosylated proteins as compared to the respective tunicamycin-treated proteins might be due to the *N*-acetylglycosamine residues which are left asparagine-linked (Tarentino *et al.*, 1985). Our experiments, however, did not result in a complete reduction of the M_r of the three glycoproteins to the respective values predicted from nucleotide sequence data for the *env* gene products. Other post-translational modifications may account for the remaining difference (compare Allan *et al.*, 1985; Montagnier *et al.*, 1985). In summary, our results suggest the existence of three glycosylated HFV-specific polypeptides (gp170, gp130 and gp47). By analogy to other retroviruses we suggest that these glycoproteins represent HFV *env* gene products, namely the *env*-encoded precursor, surface protein and transmembrane protein, respectively. In [^{35}S]methionine-labelled lysates of pelleted virus, only gp130 and not gp170 seemed to be present, and gp47 constituted one of the major antigens, thereby adding support to this view. However, findings of Cavalieri *et al.* (1981) and Benzair *et al.* (1985) for the closely related simian foamy virus type 1 (SFV-1; Mergia *et al.*, 1990), are in contrast to our results; they described two presumed SFV-1 *env* proteins, gp70 (surface protein) and p30 (transmembrane protein), respectively. We failed to identify a glycosylated HFV-specific protein in the range of 70K.

Next we applied RIPA to identify foamy virus antibody-positive sera of simian origin taking advantage

of the broad cross-reactivity among foamy viruses in serological assays (Nemo *et al.*, 1978; Hooks & Detrick-Hooks, 1981). As shown in Fig. 4, nine out of 12 sera reacted positively with HFV antigen (T2 to T4, T8 to T13). The presumed surface glycoprotein gp130 was clearly recognized by four out of nine positive sera (T3, T4, T12 and T13). To some extent, however, it also appeared non-specifically with negative sera (T5 to T7). Positive sera also contained antibodies reacting against the presumed *env*-encoded precursor, gp170, and the presumed transmembrane protein, gp47. A protein of 70K seemed to be of special diagnostic value in our test system since it appeared even with weakly reacting positive sera (macaque sera T8, T10 and T11). No non-specific protein bands were detected in this M_r range. Therefore, p70 seems to be particularly suitable for the discrimination between foamy virus-positive and -negative sera.

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