Human foamy virus infection activates class I major histocompatibility complex antigen expression

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We examined the effect of human foamy virus (HFV) infection on the expression of human major histocompatibility complex molecules. Our data show that in vitro HFV infection of U373-MG glioblastoma cells results in increased expression of class I human leukocyte antigen (HLA) and transcripts. Transient transfection assays of plasmids containing the reporter gene chloramphenicol acetyl transferase driven by different 5' deletions of the HLA-A11 class I promoter allowed identification of cis-acting elements involved in this regulation. HFV infection has two opposite effects on the HLA class I promoter: transactivation of the HLA-A11 promoter through a positive regulatory element located in the −525 to −335 region upstream of exon 1 and down-regulation of transcriptional activity driven by the −335 to −205 class I promoter region. Additional experimental data indicate that the effect of HFV on HLA class I expression is not mediated by the interferon pathway.

Foamy viruses constitute the third subfamily, Spumavirinae, of the Retroviridae. These viruses have a broad range of hosts with respect to cell types and species. In their natural hosts, foamy viruses appear to establish latency. In cell culture, they share a highly cytopathic effect, associated with the formation of giant cells and vacuoles (Hooks & Gibbs, 1975).

The complete genomes of simian and human foamy viruses (HFV) have been cloned and sequenced (Rethwilm et al., 1987; Kupiec et al., 1991; Renne et al., 1992). Like other complex retroviruses, including human immunodeficiency virus type 1 (HIV-1) and human T cell lymphotropic virus type I (HTLV-I), HFV contains, in addition to the structural genes gag, pol and env, a region with at least three open reading frames, termed bel1, bel2 and bel3, located between the env gene and the 3' (LTR) (Flügel et al., 1987). The bel1 gene product is a nuclear regulatory protein that transactivates HFV and HIV-1 LTR-dependent gene expression. Different bel1-responsive elements have recently been described in the U3 region of the HFV LTR (Lee et al., 1993). The bel1 gene has also been shown to be essential for HFV replication in vitro (Löchelt et al., 1991). The Bel1 N-terminal sequence fused to the Bel2 sequence generates a fusion protein named Bet, which has been found to be abundant in HFV-infected cells (Löchelt et al., 1991; Giron et al., 1993). No function has yet been assigned to bel3 (Weissenberger & Flügel, 1994), bet or bel2 gene products. However, by virtue of their genomic location and limited homology to certain human retrovirus regulatory genes, these proteins may have regulatory functions that qualitatively and quantitatively affect HFV gene expression as well as the expression of a number of important host cell genes or gene products such as cytokines or major histocompatibility complex (MHC) antigens. Recent results obtained in our laboratory suggest an association between spumaretrovirus molecular markers and Graves’ disease (Lagaye et al., 1992). Alteration of human leukocyte antigen (HLA) expression may play a role in initiation of the autoimmune process, as previously described (Faustman et al., 1991; Roitt et al., 1992; Tolosa et al., 1992).

Several viruses, including adenviruses (Schirer et al., 1983; Eager et al., 1985; Vasavada et al., 1986; Kralli et al., 1992), HTLV-I (Mann et al., 1983; Sonoda et al., 1987; Sawada et al., 1990) and HIV-1 (Schepper et al., 1989; Howcroft et al., 1993) have been shown to modulate host MHC antigen expression level. This is potentially important in that the host animal T cells require MHC molecules in order to respond to viral antigens. Class I MHC antigens are involved in the presentation of cytoplasmic antigens to CD8+ cytotoxic T cells and thus play a key role in the recognition and killing of virally infected cells, whereas class II MHC antigens are responsible for the binding and presentation
of circulating antigens to helper T cells (Brodsky & Guarigliai, 1991). The alteration of HLA expression at the surface of infected cells favours virus escape from immune surveillance and could be a factor in the development of autoimmune diseases (Maudley & Pound, 1991).

In this context, we decided to investigate whether HFV could alter MHC expression during acute infection in human cell lines. Human glioblastoma cells U373-MG (ATCC HTB17) were infected with HFV at a m.o.i. of approximately 0.5 p.f.u./cell. HFV-infected cells were harvested at 24 h and 48 h post-infection (p.i.).

Initially, the levels of expression of HLA class I and class II antigens on the surfaces of HFV-infected cells were quantified using monoclonal antibodies W6/32, which recognize a monomorphic determinant present at the surface of all HLA A, B and C locus products (Barnstable et al., 1978) and anti-DR against MHC class II molecules (HLA-DR; Dakopatts). After infection, cells were surface-labelled by incubating $5 \times 10^6$ cells with W6/32 or anti-DR for 30 min at 4°C. The cells were then washed with PBS containing 1% BSA. After washing, a FITC-conjugated Fab'$_2$ fragment of goat anti-mouse IgG (Cappel) was added to the cell pellet for 30 min at 4°C. The cells were washed, fixed in 1% paraformaldehyde and analysed by cytofluorography (Epic Profile; Coulter).

The cell surface expression of MHC class I and II is shown in Fig. 1. The U373-MG cell line expresses a basal level of HLA class I antigens. About 70% of cells were positive for HLA class I, as detected by W6/32 antibody, and this level was the same for cells inoculated with irradiated HFV (Fig. 1b). There is evidence of an increase in MHC class I antigen expression after HFV infection. The upshift in HLA class I on U373-MG cells occurred 24 h after HFV infection and persisted 48 h p.i. (Fig. 1b). This could be a transient phenomenon, as observed for HIV-1 (Scheppler et al., 1989), since HLA class I expression seemed to decrease later p.i. (data not shown).

As shown in Fig. 1(c), expression of MHC class II surface antigens, detectable with anti-DR monoclonal antibody, does not appear to vary significantly in the time period during which marked changes are seen in the
expression of MHC class I antigens (Fig. 1b, c). For these reasons, we further investigated HFV-mediated HLA class I up-regulation in U373-MG cells.

Total intracellular RNA was extracted from U373-MG HFV-infected cells, U373-MG cells inoculated with irradiated virus, untreated U373-MG cells and from human chronic myelogenous leukaemia K562 cells (ATCC CCL 243) which are known to express no HLA class I mRNA. The RNAs were denatured with glyoxal and fractionated on agarose gels as previously described (McMaster & Carmichael, 1977). The RNAs were transferred onto GeneScreen Plus membrane (Du Pont NEN) and probed with 32P-labelled HLA class I probe, an almost complete cDNA of the HLA B7 gene (Sood et al., 1981). After autoradiography, the probe was stripped off the membrane, which was then rehybridized with a bel-specific probe. This probe was prepared from a recombinant clone pHFV-H-C-55 (Flügel et al., 1987) and corresponds to the EcoRI–HindIII 1.2 kb fragment covering the 3′ part of the env gene and the bel region. The bel probe hybridizes to all HFV transcripts. Northern blot analysis of the mRNAs extracted from 24 h and 48 h HFV-infected U373-MG cells is shown in Fig. 2. The level of MHC class I-specific mRNA is highly increased 24 h after viral infection (Fig. 2b, lane 1) compared to that observed in untreated U373-MG cells or in U373-MG cells inoculated with irradiated HFV (lanes 4 and 5). MHC class I expression decreases 48 h p.i., to slightly under the basal level of control U373-MG cells (lane 2 and 5). As expected, no HLA class I mRNAs were detected in K562 cells (lane 3). With the HFV-specific probe, a variation in the pattern of HFV transcripts was observed during the course of infection (Fig. 2a). The high expression of HLA mRNA correlates with the time in the HFV infection cycle when early gene products, in particular Bell, begin to be expressed (Giron et al., 1993). Other viral mRNAs expressed later during this cycle may balance the effect. A glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was used as a control to quantify RNA on Northern blots and its level was found to be identical in infected and control cells (Fig. 2c).

In many systems it has been shown that regulation of expression of MHC class I genes takes place at the transcriptional level (David-Watine et al., 1990). Therefore, in order to determine whether the effect of HFV on HLA class I genes occurs at the transcriptional level and in order to map the cis-acting elements that are responsive to HFV within the HLA class I promoter, we carried out a study with a series of 5′ deletions of the MHC class I promoter-driven chloramphenicol acetyl transferase (CAT) constructs (Fig. 3a). The HLA–CAT plasmids (−525/+2; −335/+2; −205/+2) used in these experiments contained 525, 335 and 205 bp sequences, respectively, upstream from the ATG of the first exon of the HLA-A11 class I gene and included known transcriptional regulatory elements (Blanchet et al., 1994). The −525/+2 region of the HLA-A11 class I gene was introduced into the HindIII and BamHI sites of the pBLCAT3 vector (Luckow & Schütz, 1987). The −205/+2 and −335/+2 regions were prepared by PCR amplification of corresponding regions on cosmid DNA containing the HLA-A11-coding gene and flanking regions (Paul et al., 1985) and introduced between the blunted XbaI and BamHI sites of the pBLCAT3 plasmid. Construct sequences were controlled by dideoxy-nucleotide chain termination sequencing. The CAT activity of the three constructs relative to each other was
Fig. 3. (a) Structure of pHLA-CAT plasmids. The $-525/+2$, $-335/+2$ and $-205/+2$ regions of the HLA A11 class I gene were introduced upstream of the CAT reporter gene in the pBLCAT 3 promoterless vector. (b) U373-MG cells were transfected with the appropriate promoter CAT construct and either infected or not 24 h later with HFV. Values (an average of four or five duplicate samples) represent CAT activity compared to control transfections in U373-MG cells inoculated with irradiated HFV.

tested in uninfected cells. The $-525/+2$ construct has the weakest basal promoter activity (assigned an arbitrary value of 1), the $-335/+2$ is 3.5-fold more active, while the $-205/+2$ construct, which contains the enhancer A and interferon consensus (ICS) elements, shows a 6.2-fold increase in activity over the $-525/+2$ construct. Prior to transfection, cells were washed twice in serum-free medium and resuspended at a concentration of $5 \times 10^6$ cells/250 ml. The various CAT constructs (15 g) were introduced into U373-MG cells by electroporation. Transfected U373-MG cells were infected the following day. Protein contents of cell extracts were quantified using the Bio-Rad protein assay and normalized amounts of protein were used to gauge CAT activity. CAT activity was determined 24 h.p.i., as already described (Gorman et al., 1982) and quantified by liquid scintillation counting of spots cut from chromatography plates. The ratio of non-acetylated to acetylated forms was calculated, and the mean values obtained from at least four representative experiments, each consisting of two independent duplicates, were expressed relative to the mean values obtained in uninfected cells (Fig. 3b).

The promoter region of MHC class I has previously been found to contain CAAT and TATA boxes and two cis-acting elements, the class I regulatory element (CRE) and ICS (Israel et al., 1986; Kimura et al., 1986; Baldwin & Sharp, 1987, 1988). First, we explored the involvement of the main MHC regulatory cis-acting elements (enhancer A/RI or CRE and IRS/ICS) by studying the activity of a $-205/+2$ construct containing the basal promoter and the RI enhancer in transient transfection experiments. In contrast to several other agents known to regulate HLA class I, HFV showed no detectable effect on these cis-acting elements (Ge et al., 1992; van’t Veer et al., 1993).

To address whether the sequences upstream of $-205$ bp in the HLA promoter contained elements that might play a role in HFV-mediated regulation, we studied the activity of two different regions in the HLA promoter with the $-335/+2$ and $-525/+2$ bp constructs in U373-MG HFV-infected cells as well as in control cells. In U373-MG HFV-infected cells, the CAT activity of the $-335/+2$ construct was repressed about threefold, while that of the $-525/+2$ construct was enhanced around twofold (Fig. 3b). These results were highly reproducible in all our CAT assays. The activation of the $-525/+2$ region in infected cells increased linearly with the amount of transfected plasmid, whereas a faint increase of the acetylation was observed in uninfected cells, probably due to the carrier DNA effect (Fig. 4). These data indicate that HFV regulates the...
expression of HLA class I genes by affecting their transcriptional rates and that the promoter regions between -335 and -205 bp, and -525 and -335 bp are involved in down- and up-regulation respectively. The -525/+2 construct contains the regulatory elements included in -335/+2, which respond negatively to HFV infection, as well as unknown elements which participate in up-regulation of MHC class I mRNA. The region between -525 and -335 bp could represent regulatory domains required for HLA activation by HFV. The up-regulation observed with this sequence correlates with that observed in U373-MG HFV-infected cells by Northern blot and flow cytometry analysis at 24 h p.i.

Our conclusion, based on Northern blot and immunofluorescence analysis, supports the notion that the expression of endogenous MHC class I genes in U373-MG HFV-infected cells is increased at 24 h p.i. and later decreases. Previous studies have shown that other viruses have an effect either on constitutive or on virus-induced host cell MHC class I gene expression (Schrier et al., 1983; Scheppeler et al., 1989; Sawada et al., 1990). Data from the literature suggest that MHC class I expression might be modulated via several distinct mechanisms of regulation. Lymphokines and viruses are known to influence the expression of these molecules at the cell surface in vivo and in vitro (Yoshie et al., 1982; Rosa et al., 1983, 1986; Halloran et al., 1986).

One explanation of the effect of HFV on HLA class I expression could be an indirect increase in the transcription of MHC I genes by an autocrine mechanism, i.e. by up-regulating interferon in the infected cells. However, this pathway seems not to be involved in the HFV effect because no increase in 2′5′ oligoadenylate (2′5′A) synthetase activity was observed in infected cells compared to the control cells and, in addition, the supernatants of U373-MG HFV-infected cells had an interferon titre value below the detection limit and did not induce any change in enzyme levels in WISH cells (Table 1). These results clearly indicate that interferon is not secreted upon HFV infection and they correlate with those obtained from transfection assays using the -205/+2 construct, which contains the interferon responsive element but was unaffected by HFV infection.

The reduced rate of CAT activity obtained with the -335/+2 construct could correspond to lower HLA class I enhancer activity in U373-MG HFV-infected cells compared to that observed in control cells. Elevated levels of a repressor protein in HFV-infected cells might compete with a positive transcription regulator for DNA binding. We observed that additional mechanisms of cis-acting sequences located between -525 and -335 bp are involved in HFV-mediated MHC class I regulation. The results presented here for CAT assays with the -525/+2 construct indicate that a distal 200 bp sequence (-525/-335 bp) mediates the activation of the promoter activity by HFV. Interestingly, we observed two opposite effects: an up-regulation involving the -525 to -335 bp region and a down-regulation involving -335 to -205 bp. This latter effect is only visualized in the absence of the up-regulating sequence.

The results obtained with HLA class I RNAs and proteins in U373-MG-HFV infected cells 24 h and 48 h p.i. may be due to a combination of positive and negative regulations. In both types of modulation, HFV proteins may be involved in regulating HLA class I antigen expression. In particular, the early gene products of HFV, such as Bell, could play a role in this regulation, as suggested by our preliminary results in transfection assays indicating an up-regulation of HLA class I with a bell expression vector. A somewhat similar situation is seen in glial cells transfected with HTLV-I transactivator, in which Tax induces transcriptional activation of the MHC class I genes. This latter modulation is not mediated by any soluble factors, such as interferons (Sawada et al., 1990). Recently HIV-1 Tat protein has been shown to repress MHC class I gene promoter activity, but the mechanism involved in this regulation is not known (Howcroft et al., 1993). One or more HFV products may play a role in MHC class I modulation as

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<tr>
<th>2′5′A synthetase (units/mg)</th>
<th>Interferon titres* (units/ml)</th>
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<td>U373-MG cells*</td>
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<tr>
<td>Irradiated HFV-treated cells</td>
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<td>HFV-infected cells</td>
<td>20</td>
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<td>Human interferon β</td>
<td>ND</td>
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* U373-MG cells were challenged with HFV or irradiated HFV and 2′5′A synthetase activity was determined after 24 h as previously described (Justesen et al., 1980; Chelby-Alix et al., 1991).
† Supernatants of U373-MG cells were tested for their capacity to induce 2′5′A synthetase activity in WISH cells.
‡ Interferon titres are expressed relative to the human interferon β reference of the NIH, USA.
ND, Not determined.

Table 1. Interferon and 2′5′A synthetase activities in the extracts and supernatants of HFV-infected U373-MG cells
observed in the case of adenovirus C-transfected cells, in which the E1A protein enhances the effect of E3 on class I MHC antigen expression (Routes et al., 1993).

Infection and inappropriate HLA antigen expression could result in triggering of polyclonal T and B cell immune responses and induction of autoaggressive immune effectors in vivo. The role of the deregulation of MHC gene expression in HFV-infected cells remains to be investigated further.

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