The effect of the cellular stress response on human T-lymphotropic virus type I envelope protein expression

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In this report the influence of the cellular stress response in mediating changes in human T-lymphotropic virus type I (HTLV-I) viral envelope (Env) protein metabolism is determined. Previously, we reported that induction of the cellular stress response enhanced HTLV-I-mediated syncytia formation following induction of the cellular stress response in persistently infected lymphocytes. In this study, we show that the increase in HTLV-I-mediated syncytia formation following stress response induction is due to a result of increased cell surface expression of viral Env protein (gp46). Cellular stress in MT 2.6 cells did not alter the turnover of intracellular Env protein (gp68) as no changes in viral protein half-life were demonstrated as compared to non-stressed cells. However, Env expression in stressed cells treated with a protein synthesis inhibitor (cycloheximide) indicates the effect is mediated through increased translation of viral Env protein.

Surface expression of human T-lymphotropic virus type I (HTLV-I) envelope (Env) protein is essential for the syncytia forming ability of infected cells (Nagy et al., 1983). Consequently, syncytia formation assays are frequently used to measure changes in viral Env biological activity. HTLV-I viral Env protein is synthesized as a precursor protein (gp68), which is subsequently cleaved to a surface protein (gp46) and a transmembrane protein (gp21) (Hattori et al., 1984; Schneider et al., 1984). The precursor protein is translated from a singly spliced mRNA species on membrane (endoplasmic reticulum)-bound polyribosomes. The protein is then modified by glycosylation events, proteolytically cleaved and inserted into the host cell membrane during virion assembly and release (Paine et al., 1994; Pique et al., 1992).

The heat shock response, or cellular stress response, is a defence mechanism against conditions that are damaging to the cell and is induced by a variety of stimuli including heat shock and sodium arsenite (Nover et al., 1991). Previously, we reported that induction of the cellular stress response by sodium arsenite or thermal treatment increased syncytia formation in HTLV-I-infected cells (Andrews et al., 1995). The stress-mediated increase in syncytia formation was inhibited by incubating the cells in the presence of a monoclonal antibody against gp46. Western blot analysis revealed increased intracellular viral Env protein (gp68) expression following induction of the stress response. However, Northern blot analysis revealed mild differences in Env mRNA in response to cellular stress and the differences did not compare to the magnitude of intracellular Env protein expression and syncytia formation. This disparity suggested that in HTLV-I-transformed cell lines, the accumulation of viral Env protein in response to cellular stress is in part a result of post-transcriptional events.

The enhancement of HTLV-I-mediated syncytia formation has important implications for cell-to-cell transmission of HTLV-I. This highly cell-associated virus is primarily transmitted between virally infected cells through membrane fusion. Therefore, disease states potentially associated with stress response induction (e.g. febrile episodes associated with opportunistic infections) may enhance HTLV-I virus load within an infected person. The present study was designed to determine the effects of physiological stress on Env protein metabolism in HTLV-I-transformed cell lines.

To first determine if the increase in syncytia formation in stressed cells was a result of enhanced surface expression of the HTLV-I Env protein (gp46), we determined the expression of gp46 by flow cytometric analysis following stress induction in a clone of MT 2 cells (Miyoshi et al., 1981) derived by limited dilution (designated MT 2.6) and HUT 102 cells (Gadzar et al., 1980). The cellular stress response was induced in these HTLV-I-transformed cell lines, using sodium arsenite as previously described (Andrews et al., 1995). HTLV-I surface Env expression was determined following stress induction by indirect immunofluorescence staining of 1 x 10⁶ cells for gp46. The
primary antibody was a 1:200 dilution of a murine MAb against gp46 (IC11; Palker et al., 1989). The secondary antibody was a 1:20 dilution of a rabbit anti-mouse IgG FITC-conjugate (Sigma). All staining was performed on ice and antibodies were diluted in PBS containing 1% BSA and 0.1% sodium azide to prevent internalization of antigen–antibody complex. Following staining, the cells were fixed in 2% paraformaldehyde. Specific staining was measured by flow cytometric analysis using an Elite cytometer (Coulter). Isotypic FITC-labelled MAb controls (mouse IgG, Sigma) were included in each trial. Fig. 1(a) is a representative trial showing overlaid fluorescence histograms which demonstrates the shift in mean channel fluorescence in the stressed cells as compared to non-stressed cells at 24 h. The immunofluorescent flow cytometric data were analysed using the Immuno-4 analysis program (Coulter) to calculate the percentage of positive cells in the test histograms. The mean percentage expression of gp46 was 33% and 17% at 24 h ($n = 5$) and 48 h ($n = 3$), respectively. A similar pattern of expression was observed in HUT 102 cells (data not shown).

We performed a kinetic study to follow the expression of gp46 post-physiological stress. Cells were labelled for gp46 at 3, 6 and 24 h post-stress and expression of heat shock protein (hsp) 72 was determined simultaneously (Fig. 1b). Hsp72 is the highly inducible member of the hsp70 family, and a very sensitive indicator of an active stress response (Lindquist, 1986). Increased surface expression of gp46 was observed as early as 3 h, with peak expression at 6 h following stress induction. The expression of hsp72 followed a similar pattern as gp46; however, peak expression occurred prior to gp46 in MT 2.6 cells (Fig. 1b).

To address the effect cellular stress had on the turnover of the Env precursor protein, we determined the $t_{1/2}$ of gp68 following induction of the cellular stress response in MT 2.6 cells. A radioimmunoprecipitation assay (RIPA) was performed as previously described with some modifications (Hartley et al., 1990). MT 2.6 cells were metabolically labelled with $[^{35}S]$-cysteine and $[^{35}S]$-methionine (TransS Label, ICN) after a 20 min incubation in methionine/cysteine-free RPMI medium. After 30 min pulse-labelling, cells were washed and resuspended in RPMI medium supplemented with 10% foetal calf serum, 1-2 mM glutamine, 60 U/ml penicillin, 60 µg/ml streptomycin, and a fivefold excess of methionine/cysteine. The cellular stress response was induced by exposing the cells to sodium arsenite for 1.5 h and lysates were collected at 3, 6, 12 and 24 h following stress induction. Cells were disrupted with RIPA lysing buffer (1× PBS, 0.1% SDS, 0.5% deoxycholate, 1% Triton X-100) containing protease inhibitors and clarified for 1 h at 14 000 r.p.m. Supernatants were collected and preincubated with a 50% suspension of protein A–Sepharose beads (CL-4B, Sigma) for 60 min. The lysates were incubated with a MAb (1:20 dilution) against HTLV-I gp46 (IC11) for 2–18 h at 4 °C. Immune complexes were precipitated with protein A–Sepharose, washed with RIPA lysing buffer and eluted by boiling in 2× Laemmli sample buffer. Samples were electrophoretically resolved in an SDS–10% polyacrylamide gel and relative amounts of radio-labelled proteins determined with a Molecular Dynamics PhosphorImager. The $t_{1/2}$ was calculated as $\ln(N_{o}/N)/\ln(2)$, where $N_{o}$ represents the relative amount of pulse sample and $N$ represents the relative amount of chase sample (Luscher & Eisenman, 1988). Pulse–chase experiments determined that the turnover of gp68 in non-stressed cells had a $t_{1/2}$ of 9–14 h (Fig. 2a, b). This compared to a previous study reporting a $t_{1/2}$ of 7–12 h for gp68 in MT 2 cells (Paine et al., 1994). Pulse–chase experiments in stressed cells revealed a similar $t_{1/2}$ of 13 and 14 h, respectively (Fig. 2a, b), therefore indicating minimal change in gp68 turnover in stressed MT 2.6 cells.

To determine the stress-mediated effect on viral Env synthesis at the translational level, stressed and non-stressed cells were incubated in the presence of cycloheximide, a protein translation inhibitor, to evaluate decay of steady-state gp68 in the absence of new protein synthesis. Western blot analysis was performed for detection of HTLV-I envelope...
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Fig. 2. Effect of cellular stress on HTLV-I Env (gp68) protein turnover. (a) MT 2.6 cells were pulsed-labelled with [35S]methionine and [35S]cysteine, and chased after induction of the cellular stress response with sodium arsenite treatment. Cell lysates from non-stressed and stressed cells were immunoprecipitated with anti-gp46 MAb. (b) Graphical representation of the degradation of gp68 shown in (a). This demonstrates the similar turnover of gp68 in stressed cells (unbroken line) as compared to non-stressed controls (broken line).

Fig. 3. Effect of cellular stress on the translation of HTLV-I Env (gp68) protein. MT 2.6 cells were stressed with sodium arsenite, then cycloheximide (100 µg/ml) was added. The expression of gp68 was determined 24 h later by Western blot analysis. NS, non-stressed; S, stressed.

glycoproteins and hsp72. MT 2.6 cells were lysed and solubilized in lysis buffer (6 ± 25 mM Tris–HCl, pH 6 ± 2, 2% SDS, 10% glycerol, 50 mM DTT), separated on an SDS–10% polyacrylamide gel and transferred to nitrocellulose, as described (Andrews, 1995). HTLV-I gp68 or hsp72 was visualized by enhanced chemiluminescence following the manufacturer’s recommendations (ECL, Amersham Life Science). Cycloheximide (Sigma) was added to selected cultures at a final concentration of 100 µg/ml to inhibit protein synthesis. In stressed, cycloheximide-treated cells the rate of protein synthesis (gp68) was decreased to 35% of control (stressed, non-treated) cells (Fig. 3). In addition, increased expression of gp68 was observed following stress induction. Non-stressed, cycloheximide-treated cells decreased the rate of protein synthesis to 49% of control (non-stressed, non-treated) cells (Fig. 3). Therefore, we conclude that the increased expression of gp68 following stress induction is primarily a result of increased protein synthesis and not due to changes in protein turnover (t½).

Our data indicate that the increased expression of gp68 following stress induction is a result of viral protein synthesis at the translational level and not enhanced stability of steady-state gp68. Previously, we reported an enhancement of basal gene transcription following induction of the cellular stress response (Andrews et al., 1997). We also observed there was a difference between the magnitude of stress-induced change of viral gene expression in HTLV-I-transformed cell lines, in that the amount of intracellular Env generally was two- to threefold greater than the increase in viral RNA transcripts, suggesting a post-transcriptional event (Andrews et al., 1995). In this study, to determine the half-life of gp68, MT 2.6 cells were metabolically labelled following stress induction. We demonstrate that there is no difference in gp68 turnover in stressed cells as compared to non-stressed controls. However, when protein synthesis was inhibited by cycloheximide and the amount of gp68 was determined following stress induction, we found there was no increase in expression of gp68 in the stressed cells as compared to controls. This, indicates that the stress-mediated increase in Env expression (over basal transcription) in HTLV-I-transformed cell lines is likely a result of increased synthesis due to increased translation.

Induction of the cellular stress response induces the expression of a set of proteins referred to as hsp, or cellular stress proteins (Lindquist, 1986). Following physiological stress, there is increased transcription and translation of hsp with decreased synthesis of most other cellular proteins (Panniers, 1994). This preferential pattern of protein synthesis is to promote survival of the cell following stress induction. The mechanism of translational control is not completely understood; however, it appears that there is suppression of translation of non-hsp mRNA rather than degradation (Storti et al., 1980). One major role of regulation is inhibition of polypeptide chain initiation (Panniers & Henshaw, 1984;
Hickey & Weber, 1982; McKenzie et al., 1975). There is also evidence that elongation is another mechanism of translational regulation of hsp messages (Ballinger & Pardue, 1983; Theodorakis et al., 1988), in that ribosomes elongate faster on hsp mRNA following stress compared to non-stressed cells. One study evaluating the turnover of c-myc and c-myb following thermal stress demonstrated not only an increase in the half-life of c-myc (five- to eightfold) and c-myb (twofold) but also increased protein synthesis (twofold) of c-myc at the translation level (Luscher & Eisenman, 1988). This resulted in an increase in c-myc protein expression following thermal stress. The reason for the selective synthesis and increased turnover of the c-myc protein is unknown, but it is speculated that there is either a need for the protein during stress recovery or for the reintiation of proliferation.

We recognize that the stress response may be modulating replication at multiple levels (i.e. transcription and/or translation); however, our previous data (Andrews et al., 1995, 1997) revealed stress-mediated changes in basal transcriptional rates could not explain the degree of enhancement of intracellular viral protein expression. Although the precise mechanism is unknown, we speculate that the increase in synthesis of HTLV-I Env protein may be translationally controlled in a similar way to hsp following stress induction (e.g. preferential mRNA translation). Biologically, this may provide an escape mechanism for virion release from a damaged cell or perhaps a protective cellular mechanism to allow enhanced recognition of the highly immunogenic Env protein.

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References


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