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The unfolded protein response in hereditary haemochromatosis

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Abstract

To cope with the accumulation of unfolded or misfolded proteins the endoplasmic reticulum (ER) has evolved specific signalling pathways collectively called the unfolded protein response (UPR). Elucidation of the mechanisms governing ER stress signallinghas linked this response to the regulation of diverse physiologic processes as well as to the progression of a number of diseases. Interest in hereditary haemochromatosis (HH) has focused on the study of proteins implicated in iron homeostasis and on the identification of new alleles related with the disease. HFE has been amongst the preferred targets of interest, since the discovery that its C282Y mutation was associated with HH. However, the discrepancies between the disease penetrance and the frequency of this mutation have raised the possibility that its contribution to disease progression might go beyond the mere involvement in regulation of cellular iron uptake. Recent findings revealed that activation of the UPR is a feature of HH and that this stress response may be involved in the genesis of immunological anomalies associated with the disease. This review addresses the connection of the UPR with HH, including its role in MHC-I antigen presentation pathway and possible implications for new clinical approaches to HH.

Keywords: chemical chaperones • hereditary haemochromatosis (HH) • HFE • major histocompatibility complex class-I (MHC-I) • unfolded protein response (UPR)

HFE

HFE is a member of a rather large family of proteins sharing common structural features, which are encoded by a group of genes located on the MHC region in chromosome 6. The protein is a 343 residue type I transmembrane glycoprotein homologous to class I major histocompatibility complex (MHC-I)

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molecules (such as HLA-A2, with which it shares 37% sequence identity) [1]. The extracellular portion of HFE consists of α1 and α2 domains and an immunoglobulin-like α3 domain. As for the classical MHC-I proteins, HFE folding occurs in the endoplasmic reticulum (ER), where its α3 domain interacts with the class I light chain β2-microglobulin (β2m) [1]. The heterodimer is then capable of leaving the ER through the standard secretory pathway (Fig. 1). However, one significant structural difference exists between HFE and MHC-I: the putative peptide-binding groove of HFE is too narrow to accommodate a short peptide. The surface area of the groove formed by the α1 and α2 domains is approximately 415 A^2 compared to approximately 760 A^2 in MHC-I molecules. Given this structural limitation and despite its homology with MHC-I, HFE does not present antigens to T lymphocytes [1].

HFE is predominantly found in the duodenum, liver, pancreas, placenta, kidney, macrophages and ovary, while in colon, leukocytes, brain and lung it is present in lower concentrations [2].

The C282Y mutation

In the HFE C282Y mutation a single-base transition leads to the substitution of a tyrosine for a cysteine at position 282. This provokes the disruption of the α3 domain intrachain disulfide bond, which is a mandatory pre-requisite for β2m association, intracellular transport and expression of HFE at the cell surface. As a result, the C282Y mutant protein does not fold correctly and fails to bypass the endoplasmic reticulum (ER) quality control mechanisms being trapped in this compartment and subjected to premature proteasomal degradation [3, 4] (Fig. 1). Recently it was observed that this mutant protein forms intracellular aggregates *in vitro* [5]. The formation of protein aggregates caused by destabilization of the α-helical structure with simultaneous formation of a β-sheet is a common feature of almost all diseases of protein conformation [6]. These aggregates tend to resist degradation and accumulate in inclusion bodies [7] and, although their identity and contribution to disease progression are largely unknown, collected evidence suggests that they may be toxic [8].

Another common HFE mutation, the H63D mutation, whose pathogenic significance is still uncertain, involves the substitution of a histidine by an aspartate at position 63. This mutation occurs in the α1 domain of the protein and does not perturb β2m association or intracellular localization of the HFE protein [2, 9].

HFE and hereditary haemochromatosis

Hereditary haemochromatosis (HH) is the most common inherited disease in Caucasians. In these patients, intestinal iron absorption and iron export from macrophages are abnormally elevated resulting in progressive tissue iron overload which leads to irreversible organ damage if not treated timely [10]. The pancreas, heart, skin, joints and particularly the liver are the main deposition sites where the iron surplus leads to organ failure through the production of oxidant species. Cirrhosis, hepatocellular carcinoma, cardiomyopathy, diabetes and arthritis are some of the pathologies associated with chronic iron overload [11]. Nevertheless, if started before the onset of irreversible organ damage, therapeutic phlebotomy – the mainstay of the treatment of HH – offers these patients a life expectancy similar to that of a normal population.

The HFE C282Y mutation and HH

In as much as the MHC-I-like proteins are typically involved in immunological processes, such as MHC-I antigen presentation to $CD8^+$ T lymphocytes, it would seem unlikely that one of its family members is involved in the iron metabolism. However, a number of findings have related the immune system to iron overload. Nevertheless, the first clues about the identity of the gene implicated in HH were provided some time before the discovery of HFE. In 1994 it was found that the β2m knockout mice develop iron overload with a tissue distribution indistinguishable from that observed in HH [12]. This raised the possibility that the gene product defective in HH might have a structure similar to MHC-I molecules and an analogous requirement for β2m association for proper folding and function. In fact, the mapping and cloning of the human HFE revealed its sequence homology to MHC-I molecules, in particular to HLA-A2 [2].

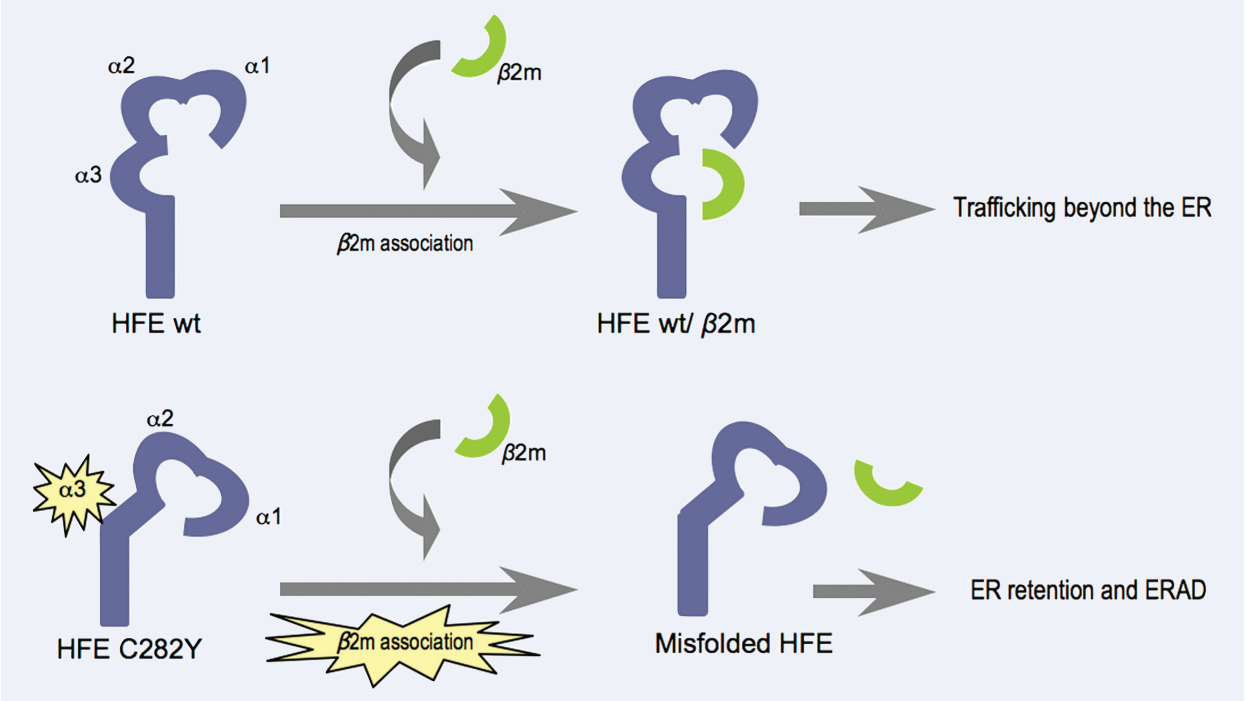

Fig. 1 Impact of the C282Y mutation on the HFE/β2m association. In the endoplasmic reticulum (ER) lumen, HFE wt proteins assemble with β2m and exit the ER through the standard secretory pathway. The C282Y mutation abrogates the formation of the α3 domain impeding the association of HFE with β2m. The resulting protein is retained in the ER and subjected to degradation.

Furthermore, the predicted HFE structure revealed that β2m association was a pre-requisite for the correct protein maturation [1] and its involvement on iron metabolism was consistent with the iron overload found in the β2m knockout mouse [3, 13].

The finding that over 83% of the cases of HH are associated with the C282Y mutation has established HFE as a key partaker in the regulation of iron homeostasis and in the progression of HH [2]. In agreement with a role in iron homeostasis and with the pathogenesis of the disease, high levels of HFE mRNA were found in tissues involved in iron mobilization and storage, like the duodenum, placenta, macrophages and the liver [14]. Adding to the evidence of an implication in iron metabolism, shortly after its discovery HFE was found to associate with the transferrin receptor 1 (TfR1) [15] and to decrease its affinity for iron-bound transferrin by 5 to 10-fold [16]. The C282Y mutation disrupts this association, thus preventing HFE's inhibitory role on iron uptake [16]. Besides this impairment of iron uptake, HH is also characterized by defects of the iron export machinery and poor iron storage in reticuloendothelial cells. HFE blocks cellular iron export from macrophages a function lost in HH because of the C282Y mutation [17].

HFE's role in the regulation of cellular iron stores and, more significantly, the impact of the C282Y mutation on this task definitely implicated this particular mutation on the physiopathology of HH.

Immunological abnormalities of HH patients

New aspects of HFE's biology are emerging suggesting that its function might go beyond the exclusive participation in iron homeostasis toward a putative function in other processes. Studies of immunological aspects of HH patients have opened the door to an unsuspected role of HFE in the immune system. Analysis of the T lymphocyte numbers showed that in

comparison to a healthy control population, HH patients exhibit higher $CD4^+/CD8^+$ ratios [18]. This was found to result from abnormally lower $CD8^+$ cells and not higher $CD4^+$ numbers [19]. Moreover, a correlation was established between this parameter and the clinical expression of HH: a lower $CD8^+$ T lymphocyte population is associated with a more severe form of the disease [20]. In addition to the T lymphocyte population's discrepancies, functional studies revealed that, in comparison to healthy controls, $CD8^+$ T lymphocytes from HH patients exhibit a diminished cytotoxic activity [21] and decreased CD8-p56lck activity [22]. This abnormality was not corrected by the phlebotomy treatments suggesting that it may be linked to aspects associated with HH other than the iron-mediated toxicity. One candidate might be HFE itself, particularly the impact of the C282Y mutation on a putative iron-independent function.

The virus connection: hints for an extended role of HFE

Two independent studies have provided mechanistic evidence for a direct effect of human cytomegalovirus (HCMV) and human immunodeficiency virus (HIV-1) on HFE expression. Upon HCMV infection, US2 protein targets HFE for proteasomal degradation interfering with its expression on the cell surface [23]. Similarly, Nef (an HIV-1 protein important for the progression to AIDS) reroutes HFE to a perinuclear compartment causing a 90% reduction of cell surface expression [24]. This way, viruses gain access to an increased intracellular iron pool, which might favour their replication [24, 25]. Moreover, a putative role in immunological processes would certainly contribute to place HFE under virus threat. In fact, the capacity of viruses to escape recognition by the immune system has led to the identification of several proteins targeted by an intricate virus-derived machinery. MHC-I is amongst the preferred targets and its decisive role in denouncing invasion of pathogens to the immune system definitely supports it.

The MHC-I antigen presentation pathway

Following infection, some pathogens assault the host cells establishing themselves in the sheltered intracellular milieu. This presents the immune system with the continuous challenge of trying to spot and destroy the invading species. However, centuries of a co-evolutionary history have produced an increasingly fine-tuned host immune response, equipped and eager to fight the infection. In this hide-and-seek relationship, the pathogens seek to gain control of one particular piece of the host's defensive arsenal: the MHC-I antigen presentation pathway. Viruses represent probably the most prominent example of this effort to overcome the host's cellular defences.

From fish to humans, all nucleated cells constitutively process and present short peptides derived from endogenously synthesized proteins. These peptides snug in the binding groove of MHC-I molecules that, after moving to the cell surface, enable $CD8^+$ T lymphocytes to recognize intracellular pathogens, such as viruses.

Designing a peptide loaded MHC-I protein

Although conceptually simple, the aim being to display a fingerprint of the intracellular content at the cell surface by means of small protein fragments, antigen presentation is a rather sophisticated process involving a number of crucial events: MHC-I folding and maturation, peptide generation and shuttle into the ER, assembly of the peptide loading complex (PLC), trafficking of peptide loaded MHC-I to the cell surface and ultimately its recognition by $CD8^+$ T lymphocytes (Fig. 2).

MHC-I antigen presentation is initiated in the ER where folding of MHC-I molecules takes place. This is a complex process, which involves the participation of a variety of auxiliary factors, like heavy-chain binding protein (BiP – also termed GRP78), the lectin chaperones calnexin and calreticulin, and the oxidoreductase ERp57 [26]. Following translocation into the ER *via* the Sec61 protein complex, MHC-I heavy chains (HC) bind to the chaperones BiP and calnexin and associate non-covalently with the soluble β2m [27, 28]. Upon β2m association, calnexin is replaced by calreticulin and the heterodimer is recruited to a multi-subunit structure, collectively termed the PLC, containing, at least, tapasin, calreticulin, ERp57 and the transporter associated with antigen presentation (TAP) [29].

The newly generated peptide-receptive MHC-I molecules are recruited into the PLC where they are stabilized until peptide loading takes place. A heterodimer

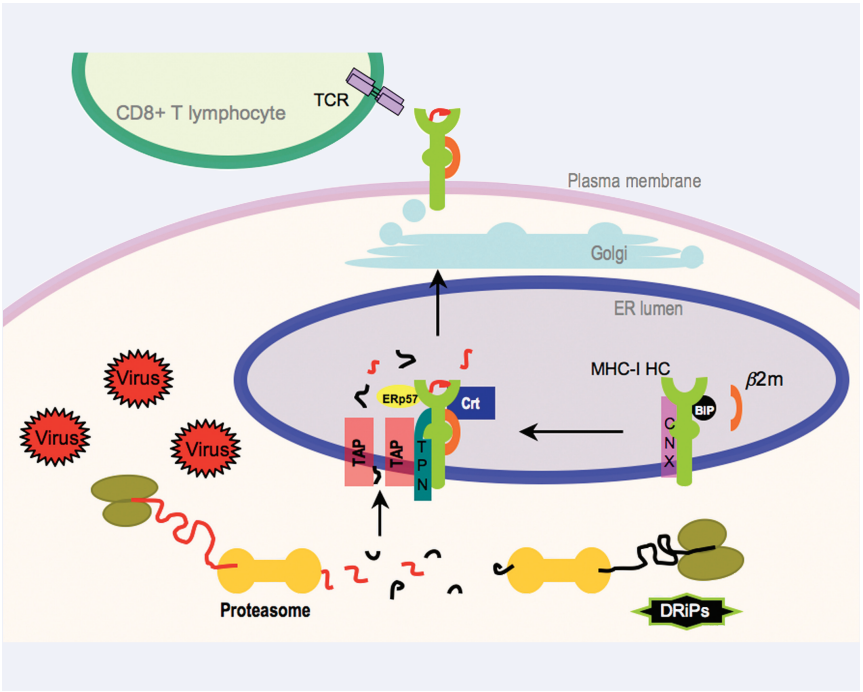

Fig. 2 The MHC-I antigen processing and presentation pathway. In the ER lumen, newly synthesized MHC-I heavy chains (HC) assemble with β2m. The interaction with the peptide loading complex (calreticulin, Erp57, tapasin and TAP) promotes peptide loading onto MHC-I molecules. The peptides are derived from endogenous antigens (including viral-derived proteins, represented in red), which are degraded by the proteasome into short peptides and transported into the ER lumen by TAP. The peptide loaded MHC-I complexes proceed to the cell surface *via* the standard secretory pathway, for presentation to $CD8^+$ T lymphocytes. CNX, calnexin; Crt, calreticulin; TPN, tapasin; DRiPs, defective ribosomal products; TCR, T-cell receptor.

of two membrane-spanning molecules, termed TAP1 and TAP2 translocates the peptides from the cytosol to the ER lumen, where loading onto MHC-I molecules takes place [30, 31]. This process favours the production of stable MHC-I/peptide complexes [32]. The action of tapasin, another component of the PLC, is thought to play a decisive role in the formation of low off-rate MHC-I/peptide complexes. Two lines of evidence provide a rationalization for this: the first is that tapasin edits the peptide repertoire in order to assure optimal MHC-I peptide loading [33]; the second explanation suggests that tapasin facilitates the binding of a more diverse set of peptides by stabilizing the MHC-I peptide-receptive conformation, but does not function as a peptide editor to discriminate between low and high-affinity peptides [34]. The definition of the precise mechanisms by which tapasin partakes on the assembly of low off-rate MHC-I/peptide complexes is still a matter of debate. Nevertheless, its crucial involvement in this process is supported by the observation that the lack of tapasin leads to a reduced stability of the MHC-I complexes at the cell surface [27–31]. The hypothesis that tapasin may not act alone in peptide loading optimization was recently supported by the finding that protein disulfide isomerase (PDI), another component of the PLC, stabilizes a peptide-receptive conformation of MHC-I by regulating the oxidation state of the disulfide bond in the peptide binding groove [35].

MHC-I molecules that fail to acquire high-affinity peptides exit the ER from a site distinct from that used by high-affinity MHC-I/peptide complexes, and are subjected either to degradation or short term expression at the cell surface followed by endocytosis and degradation [36]. MHC-I molecules, once loaded with high-affinity peptides, dissociate from the PLC, leave the ER at specific exit sites, transit through the Golgi apparatus, and emerge at the cell surface [37]. Here, they are available for scrutiny by circulating $CD8^+$ T lymphocytes that obtain access to a detailed snapshot of the intracellular environment. Eventually, if a strange (non-self) peptide is spotted, a cytotoxic immune response against the infected cell is initiated.

The source of the MHC-I peptides

Protein turnover is a common feature of all organisms. Sooner or later, all proteins are degraded as a way of controlling their properties and their relative levels. This constant supply of protein fragments

might satisfy the entire antigen presentation pathway needs and, since all proteins will eventually face proteasomal degradation, it allows for a vast and truthful portfolio of the intracellular content to be displayed by MHC-I molecules. However, protein turnover is a rather slow process. The half-life of some proteins can exceed 1 week and, in average, 1–2 days are needed for a protein to start the recycling process [38]. Since viruses can generate their progeny in few hours after infection, a distinct mechanism must operate in the host cells to assure detection of pathogens with alacrity. In fact, a shortcut between translation and peptide generation guarantees the efficiency and promptness of antigen presentation: defective ribosomal products (DRiPs) are the major source of antigenic peptides for MHC-I presentation [39].

DRiPs consist of viral and cellular proteins that fail to acquire a native conformation due to errors in transcription, translation, folding, intracellular trafficking or assembly. The findings that blocking protein synthesis with cycloheximide reduces peptide translocation by TAP and MHC-I export from the ER to the same extent as proteasome inhibition have opened the door to the theory that the major substrates for peptide generation are derived from newly synthesized proteins [40, 41]. Moreover, it was shown that inhibition of protein synthesis impairs MHC-I processing specifically by depleting the pool of rapidly degraded proteins (t1/2 of ~10 min), such as DRiPs, and that MHC-I peptides are preferentially generated from these short-lived proteins [42].

Unlike protein turnover, degradation of DRiPs offers a prompt collection of peptides for MHC-I loading. This close link between translation and antigen presentation allows a proficient immune response in situations where time is crucial like those following acute virus infections, in which efforts must rush to diminish viral replication and transmission.

Hijacking MHC-I: viruses take control

The fact that the main source of peptides for MHC-I scrutiny are DRiPs enables the immune surveillance mechanisms to detect pathogens that employ the host cell for protein synthesis [40, 43]. After infection, viruses reprogram the host's machinery to synthesize viral proteins, which are produced at very high levels. As a result, the complexity of the MHC-I peptide repertoire is biased toward peptides of viral origin [43]. To deal with this apparent limitation, several viruses have evolved sophisticated strategies to prevent the generation or MHC-I presentation of antigenic peptides, causing the immune system subversion, assuring a lifelong persistence and repeated reactivation within the host. Basically all MHC-I antigen presentation pathway events are targeted and MHC-I itself is a preferential target for a number of different viruses attempting to evade immune recognition [44, 45].

HFE C282Y influences the MHC-I antigen presentation pathway

Even though previous findings strongly suggested an extended role related to the immune system, evidence for a direct impact of HFE on a classical immunological process was described only recently. A thorough analysis of the MHC-I antigen presentation pathway revealed the existence of significant differences between HFE C282Y mutant cells obtained from HH patients and HFE wt cells. Several corrupted intracellular events lead to the generation of high off-rate MHC-I/peptide complexes that dissociate prematurely during their journey towards the cell surface. The outcome is a diminished surface expression of MHC-I molecules in HFE C282Y mutant cells [46]. Although not yet tested, the key role exerted by MHC-I molecules in a number of processes suggests that this defect may have a considerable physiological significance. In view of that, a correlation between this observation and the recognized immunological defects linked to HH may be discussed.

Signallingby MHC-I/peptide complexes determines the nature of the $CD8^+$ T lymphocytes immune response. It is reasonable to propose that impaired expression of these complexes in the context of HH may constitute the molecular basis for the diminished $CD8^+$ T lymphocytes' cytotoxic activity observed in HH patients [21]. Likewise, this would lead to decreased CD8-p56lck activity, which is also acknowledged as a feature of HH [22]. The importance and physiological significance of the interaction between the TCR in $CD8^+$ T lymphocytes and MHC-I molecules extends beyond the mere immune surveillance mechanism, playing a decisive role in processes such as the maintenance of the $CD8^+$ T lymphocytes pool, the shaping of the TCR repertoire or the natural killer (NK) cells activity [47–51].

Interestingly, a decreased pool of $CD8^+$ T lymphocytes and altered TCR repertoire are common features associated with HH [18, 19, 52]. It is tempting to suggest that these defects arise from the incapacity of HFE C282Y mutant cells to correctly process and display MHC-I at the cell surface.

MHC-I plays an inhibitory role in the shaping of the NK cells' response. An insufficient signallingby MHC-I-specific inhibitory receptors present on NK cells triggers their activation and cytotoxicity against cells that are either MHC-I negative or deficient [51]. The decreased cell surface expression of MHC-I molecules in HFE mutant cells makes them suitable candidates for targeting by NK cells. There is evidence that down-regulation of MHC-I on resting lymphocytes was sufficient to make them susceptible to NK cell killing [53]. HFE itself does not inhibit or activate the activity of NK cells [54], but by affecting MHC-I expression, HFE C282Y mutant cells may become particularly suitable targets of NK cytotoxicity.

In another study performed with transgenic mice, it was observed a direct cytolytic recognition of human HFE by mouse T cell receptors (TCR), which occurs independently of HFE-bound peptides [55]. This new function of HFE is in agreement with the previous finding that TCR-delta knockout mice develop hepatic iron overload suggesting that cellular iron status might be transmitted to lymphocytes through HFE engagement with the TCR [56].

The findings that mouse TCR directly recognize human HFE independently of HFE-bound peptides [55] and that HFE C282Y impacts on the MHC-I expression [46] provided evidence for the involvement of HFE on classical immunological mechanisms. In fact, with the exception of HFE and zinc-β2-glycoprotein [57–59], all the non-classical MHC-I molecules have previously been shown to be directly involved in immunological processes [60].

Collateral damages or selective interference? The UPR effect on MHC-I expression

The importance of an efficient folding machinery and ER quality control mechanisms is supported by the existence of an exclusive signal transduction pathway that reacts to the accumulation of unfolded or misfolded proteins in the ER: the unfolded protein response (UPR). Under certain circumstances, for instance due to increased folding demands (*e.g.* B cell differentiation into Ab secreting plasma cells), genetic mutations, infection by viruses, glucose starvation or hypoxia, the ER folding machinery and/or ER-associated degradation (ERAD) components are flooded by faulty proteins [61, 62]. To cope with this burst of 'unwanted' ER clients, the UPR is activated enhancing the levels of proteins that participate in folding, ER quality control and ERAD and reducing the rate of protein synthesis. Eventually, if the folding errors in the ER can no longer be overcome, the UPR may induce pro-apoptotic programs [63] (Fig. 3).

By preventing HFE association with β2m, the C282Y mutation causes the ER retention of HFE [4, 9]. Hypothetically, accumulation of this faulty protein in the ER proceeds until such point when its levels rise above threshold leading to the activation of the UPR. In fact, using a cell line model system genetically manipulated to express either HFE wt or HFE C282Y, higher levels of specific UPR markers were observed in those cells expressing the mutant protein [64]. Significantly, *ex vivo* PBMCs from HFE C282Y homozygous HH patients recapitulated this result establishing the UPR activation as a putative modifier of HH, taking into account its decisive role on the progression of several other diseases.

Considering the numerous intracellular events modified by the UPR signallingpathway, it was tempting to consider that the UPR might mediate the HFE/MHC-I cross-talk. Actually, induction of the UPR, either pharmacologically or by the transfection of HFE C282Y, results in a significant down-regulation of the MHC-I cell surface expression. Moreover, inhibition of the ER stress response in cells expressing the HFE C282Y mutant protein leads to the restoration of the MHC-I levels [64]. A strong link between the UPR and the MHC-I antigen presentation pathway defects was established. However, this association was found to prevail beyond the mere significance for the HH associated MHC-I defects. Regardless of the ER stress stimuli tested, it was noted that activation of the UPR consistently provokes a significant collapse of the MHC-I processing apparatus resulting in its cell surface expression decline [64].

The broad impact of the UPR on distinct cellular mechanisms, combined with the complexity of the antigen presentation pathway, does not make the

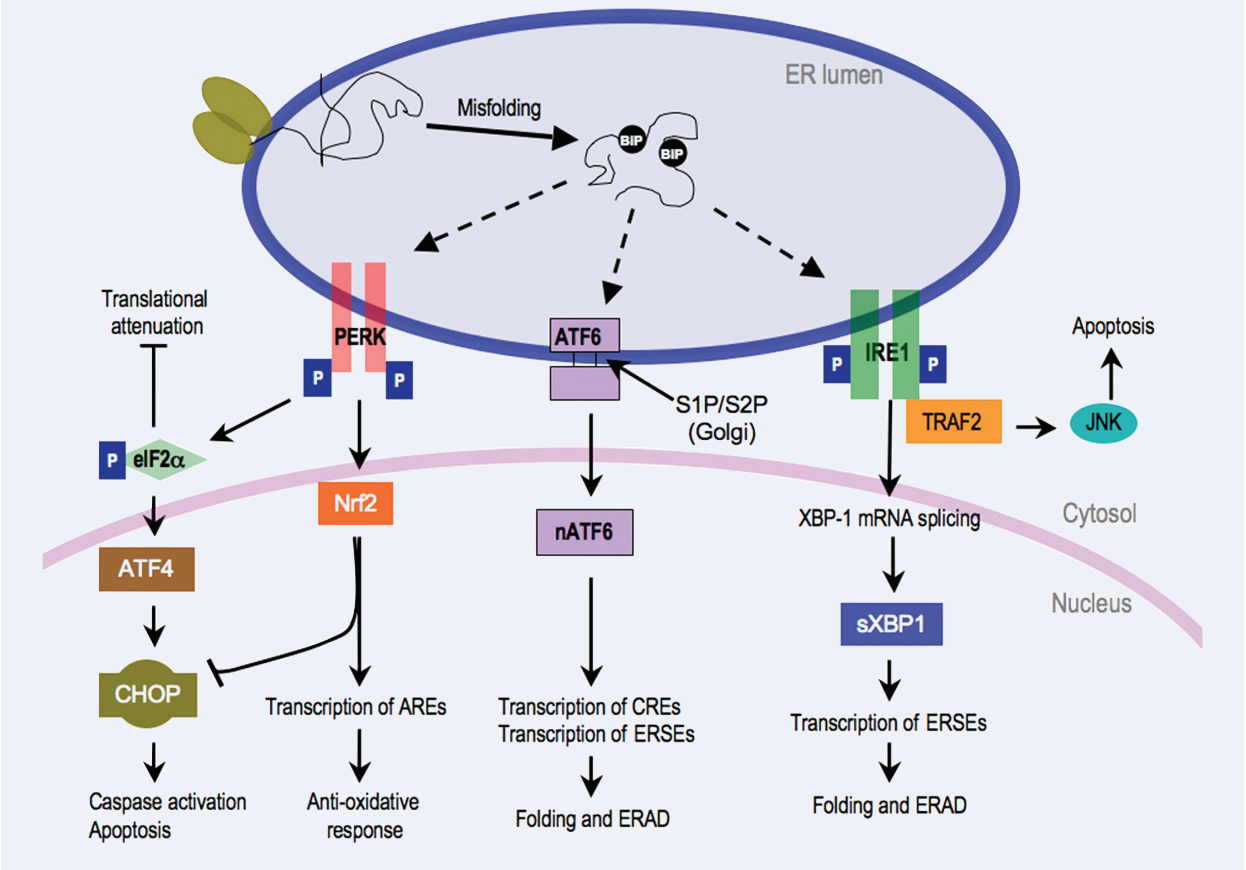

Fig. 3 The unfolded protein response in mammalian cells. Binding of BiP to unfolded proteins in the ER leads to the activation of PERK, ATF6 and IRE1. PERK activation results in eIF2α-mediated repression of general translation, but by allowing ATF4 translation, it also leads to transcriptional up-regulation of some genes, like CHOP. NRF2 phosphorylation by PERK and its translocation to the nucleus promotes the transcription of genes with antioxidant response elements (AREs) in their promoters. Activated ATF6 translocates into the Golgi where, by the action of S1P and S2P proteases, the N-terminal cytoplasmic domain of ATF6 (nATF6) is cleaved. The transcriptionally active nATF6 translocates to the nucleus increasing the transcription of genes with cyclic AMP responsive elements (CREs) or ER stress response elements (ERSEs). Activation of IRE1 leads to the alternative splicing of the mRNA for the XBP-1 transcription factor. IRE1 also binds TRAF2, activating the pro-apoptotic JNK mitogen activated kinase pathway.

establishment of a mechanistic link between the ER stress response activation and the MHC-I impairment an easy task. However, in light of some processes known to participate in both pathways, it is possible to envisage some plausible reasons for the MHC-I deficiency. As part of the struggle to relieve the folding pressure launched upon the ER, activation of the UPR promotes protein trafficking from and beyond this compartment through the induction of cargo receptors [65, 66]. The lectin ERGIC-53, a cargo receptor for the ER export of proteins and its related protein VIP36 are known targets of the UPR signallingpathway [66]. The premature removal of ER clients in situations of stress may have a significant impact on proteins relying on a dedicated quality control mechanism to achieve a stable conformation. MHC-I molecules depend on the PLC to promote the assembly of low off-rate MHC-I/peptide complexes before their journey through the standard secretory pathway [32]. This ensures that only complexes with superior stability are produced and delivered to the final destination. The extent of this peptide loading

optimization is determined by the time spent by MHC-I complexes in the ER [67] and this might be considerably shortened following UPR activation. It is therefore tempting to propose that, under stressful situations, the compulsory release of MHC-I/peptide complexes from the ER results in the production of volatile complexes that fail to reach the cell surface due to premature peptide dissociation. In agreement with this proposal, it was observed that MHC-I ER egress occurs faster in HFE C282Y mutant cells than in the wt counterparts. Furthermore, thermostability and endocytosis assays revealed a premature MHC-I/peptide dissociation in HFE mutant cells, which resulted in an increased rate of endocytosis from the plasma membrane [46].

As mentioned before, DRiPs constitute the major source of antigenic peptides for MHC-I presentation [39]. In a cellular environment where the ER folding capacity is disrupted, cells are further burdened by the continuous production of protein. To mitigate this added stress, one of the strategies employed by the UPR signallingpathway is the attenuation of protein translation by phosphorylated eIF2α [68]. However, the solid dependency of the antigen presentation pathway on fully operational transcription/translation machinery suggests a negative impact of the UPR on the MHC-I assembly. By inhibiting protein synthesis, the ER stress response might cease the most important source of MHC-I peptides, thus preventing its correct processing and cell surface expression.

Although not studied, the involvement of several molecular chaperones (*e.g.* BiP, GRP94 and calreticulin) on the UPR progression may also indirectly perturb the folding and maturation of MHC-I molecules in the ER. Moreover a possible interference on more specific partners of MHC-I, like tapasin or TAP, would also account for the antigen presentation defects observed in ER stressed cells.

Stressing out the host to escape immune surveillance

Together with a sophisticated collection of immune evasion strategies, the interference on cellular homeostasis, namely the direct targeting of the ER function, is a characteristic of several viruses. Actually, triggering of the UPR signallingpathway was already described in a number of viral infected cells [69–73]. Although the reason for this is incompletely understood, it is attractive to propose that it may constitute one of the tools that viruses have developed to escape immune surveillance. The finding that an active UPR impairs MHC-I expression [64] provides the rationale for this hypothesis. Modulation of the ER stress response would give viruses an additional tool to disrupt antigen presentation by preventing the correct trafficking of MHC-I/peptide complexes to the cell surface.

Previous studies have already insinuated an association between viral induction of the UPR and subversion of the MHC-I antigen presentation pathway on infected cells. ER stress-mediated interference on MHC-I assembly and cell surface expression was previously suggested to allow hepatitis C virus avoid detection and elimination by the immune system [74]. Differences in MHC class I expression in the liver have been reported to relate to virus genotype in patients with hepatitis C [75]. Similarly, it was observed that HCMV protein US11 provokes an UPR that may facilitate the degradation of MHC-I molecules [72]. A more detailed characterization of this link and, particularly, of the HFE C282Y-associated UPR contribution for the establishment and progression of viral infections emerges as an attractive challenge.

Pharmacological shaping of the ER stress response

By mimicking cellular molecular chaperones, which are ubiquitous stress-induced proteins, chemical and pharmacological chaperones have been found to improve the ER folding capacity and facilitate the trafficking of mutant proteins by stabilizing their conformation [76]. The therapeutic potential of these small molecular weight compounds, such as tauroursodeoxycholic acid (TUDCA) and sodium 4-phenylbutyrate (4PBA), in ameliorating the severity of a number of protein-misfolding diseases was already demonstrated. 4PBA was previously shown to increase the trafficking of a mutant cystic fibrosis transmembrane regulator [77] and to enhance the secretion of the mutant (1- antitrypsin Z protein [78]. Endogenous bile acids derivatives, such as TUDCA, can also modulate ER function protecting from UPR induction and ER stress-induced apoptosis [79, 80]. Recently, these chemical chaperones were shown to improve glucose tolerance and insulin action in a

mouse model of type 2 diabetes [80]. Moreover, TUDCA is already applied as a hepatoprotective agent in humans with cholestatic liver diseases [81, 82], while 4PBA has been in clinical use in trials for treatment of thalassaemia [83].

The finding that an UPR is activated in HH [64] has motivated the investigation of the effect of these promising pharmacologically active compounds in the modulation of the stress response. In fact, both TUDCA and 4PBA impeded the UPR activation in HFE C282Y expressing cells [5, 84]. Moreover, chemically enhancing the ER function using 4PBA also prevented the formation of intracellular protein aggregates by facilitating the degradation of HFE C282Y [5]. Since the connection between the UPR and HH is recent, additional studies are needed to elucidate the contribution of this ER stress response for the pathophysiology of the disease. Recently it was shown in liver cells that HFE C282Y enhances calreticulin mRNA expression possibly as a consequence of the UPR activation [85]. The increased levels of calreticulin were associated with reduced oxidative stress. Moreover, in HH patients, calreticulin correlated positively with the expression of the UPR marker BiP and negatively with the number of clinical manifestations [85]. The contribution of the UPR to HH progression seems to be dictated by a delicate balance between protective (*e.g.* oxidative stress) and deleterious (*e.g.* immunological anomalies) effects. A similar paradigm exists for the impact of the UPR on tumour development, in which the protective or harmful contribution of the ER stress response has been the subject of intensive study [61]. The clear elucidation of the impact of the UPR on '*iron genes*', on the iron-induced oxidative stress and the physiological significance of its associated MHC-I defects will certainly dictate the clinical value of preventing the stimulation of the UPR using chemical chaperones. The confirmed applicability of these compounds to avoid the ER stress response and of 4PBA in particular to prevent the formation of putatively toxic intracellular aggregates in HFE C282Y mutant cells stimulates further research aiming at exploring their therapeutic application in HH.

Future perspectives and concluding remarks

The discovery of the HFE C282Y-associated UPR established a link between this mutant protein and the MHC-I antigen presentation pathway [64]. Analysis of the impact of the ER stress response on other cellular processes, namely those involved in iron homeostasis, emerges as a research priority in the attempt to find a parallel between the UPR and the pathophysiology of HH. The transcriptional or translational regulation of '*iron genes*' like ferroportin, ferritin or hepcidin by the UPR signallingpathway should be further explored.

Haemochromatosis can also result from mutations in genes other than HFE, like transferrin receptor 2, ferroportin or hemojuvelin [10]. To date, activation of the UPR resulting from mutations on any of these proteins has not been described. Nonetheless, a putative involvement of the ER stress responses on non-HFE related HH demands a careful examination.

Several studies have previously aimed at drawing a correlation between HH and the progression of other diseases. It is now pertinent to dissect the putative contribution of both the HFE C282Y-associated UPR and the intracellular HFE C282Y-aggregates as a risk factor for the onset of other pathological conditions. The *in vivo* therapeutic potential of pharmacological chaperones like TUDCA or 4PBA raises the possibility that a chemical therapy for HH could replace or diminish the need for phlebotomies.

References

1. **Lebron JA, Bennett MJ, Vaughn DE, Chirino AJ, Snow PM, Mintier GA, Feder JN, Bjorkman PJ.** Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. *Cell.* 1998; 93: 111-23.
2. **Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R, Jr., Ellis MC, Fullan A, Hinton LM, Jones NL, Kimmel BE, Kronmal GS, Lauer P, Lee VK, Loeb DB, Mapa FA, McClelland E, Meyer NC, Mintier GA, Moeller N, Moore T, Morikang E, Prass CE, Quintana L, Starnes SM, Schatzman RC, Brunke KJ, Drayna DT, Risch NJ, Bacon BR, Wolff RK.** A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet.* 1996; 13: 399–408.
3. **Feder JN, Tsuchihashi Z, Irrinki A, Lee VK, Mapa FA, Morikang E, Prass CE, Starnes SM, Wolff RK, Parkkila S, Sly WS, Schatzman RC.** The hemochromatosis founder mutation in HLA-H disrupts beta2-microglobulin interaction and cell surface expression. *J Biol Chem.* 1997; 272: 14025–8.

4. **Waheed A, Parkkila S, Zhou XY, Tomatsu S, Tsuchihashi Z, Feder JN, Schatzman RC, Britton RS, Bacon BR, Sly WS.** Hereditary hemochromatosis: effects of C282Y and H63D mutations on association with beta2-microglobulin, intracellular processing, and cell surface expression of the HFE protein in COS-7 cells. *Proc Natl Acad Sci USA.* 1997; 94: 12384–9.
5. **de Almeida SF, Picarote G, Fleming JV, Carmo-Fonseca M, Azevedo JE, de Sousa M.** Chemical chaperones reduce endoplasmic reticulum stress and prevent mutant HFE aggregate formation. *J Biol Chem.* 2007; 282: 27905–12.
6. **Dobson CM.** Protein misfolding, evolution and disease. *Trends Biochem Sci.* 1999; 24: 329–32.
7. **Kopito RR.** Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol.* 2000; 10: 524–30.
8. **Caughey B, Lansbury PT.** Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. *Annu Rev Neurosci.* 2003; 26: 267–98.
9. **Pinto JP, Ramos P, de Sousa M.** Overexpression of HFE in HepG2 cells reveals differences in intracellular distribution and co-localization of wt- and mutated forms. *Blood Cells Mol Dis.* 2007; 39: 75–81.
10. **Pietrangelo A.** Hereditary hemochromatosis. *Annu Rev Nutr.* 2006; 26: 251–70.
11. **Niederau C, Stremmel W, Strohmeyer GW.** Clinical spectrum and management of haemochromatosis. *Baillieres Clin Haematol.* 1994; 7: 881–901.
12. **de Sousa M, Reimao R, Lacerda R, Hugo P, Kaufmann SH, Porto G.** Iron overload in beta 2-microglobulin-deficient mice. *Immunol Lett.* 1994; 39: 105–11.
13. **Santos M, Schilham MW, Rademakers LH, Marx JJ, de Sousa M, Clevers H.** Defective iron homeostasis in beta 2-microglobulin knockout mice recapitulates hereditary hemochromatosis in man. *J Exp Med.* 1996; 184: 1975–85.
14. **Parkkila S, Waheed A, Britton RS, Feder JN, Tsuchihashi Z, Schatzman RC, Bacon BR, Sly WS.** Immunohistochemistry of HLA-H, the protein defective in patients with hereditary hemochromatosis, reveals unique pattern of expression in gastrointestinal tract. *Proc Natl Acad Sci USA.* 1997; 94: 2534–9.
15. **Parkkila S, Waheed A, Britton RS, Bacon BR, Zhou XY, Tomatsu S, Fleming RE, Sly WS.** Association of the transferrin receptor in human placenta with HFE, the protein defective in hereditary hemochromatosis. *Proc Natl Acad Sci USA.* 1997; 94: 13198–202.
16. **Feder JN, Penny DM, Irrinki A, Lee VK, Lebron JA, Watson N, Tsuchihashi Z, Sigal E, Bjorkman PJ, Schatzman RC.** The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. *Proc Natl Acad Sci USA.* 1998; 95: 1472–7.
17. **Drakesmith H, Sweetland E, Schimanski L, Edwards J, Cowley D, Ashraf M, Bastin J, Townsend AR.** The hemochromatosis protein HFE inhibits iron export from macrophages. *Proc Natl Acad Sci USA.* 2002; 99: 15602–7.
18. **Reimao R, Porto G, de Sousa M.** Stability of CD4/CD8 ratios in man: new correlation between CD4/CD8 profiles and iron overload in idiopathic haemochromatosis patients. *C R Acad Sci III.* 1991; 313: 481–7.
19. **Porto G, Reimao R, Goncalves C, Vicente C, Justica B, de Sousa M.** Haemochromatosis as a window into the study of the immunological system: a novel correlation between $CD8^+$ lymphocytes and iron overload. *Eur J Haematol.* 1994; 52: 283–90.
20. **Porto G, Vicente C, Teixeira MA, Martins O, Cabeda JM, Lacerda R, Goncalves C, Fraga J, Macedo G, Silva BM, Alves H, Justica B, de Sousa M.** Relative impact of HLA phenotype and CD4-CD8 ratios on the clinical expression of hemochromatosis. *Hepatology.* 1997; 25: 397–402.
21. **Arosa FA, Oliveira L, Porto G, da Silva BM, Kruijer W, Veltman J, de Sousa M.** Anomalies of the $CD8^+$ T cell pool in haemochromatosis: HLA-A3-linked expansions of $CD8^+$CD28- T cells. *Clin Exp Immunol.* 1997; 107: 548–54.
22. **Arosa FA, da Silva AJ, Godinho IM, ter Steege JC, Porto G, Rudd CE, de Sousa M.** Decreased CD8-p56lck activity in peripheral blood T-lymphocytes from patients with hereditary haemochromatosis. *Scand J Immunol.* 1994; 39: 426–32.
23. **Ben-Arieh SV, Zimerman B, Smorodinsky NI, Yaacubovicz M, Schechter C, Bacik I, Gibbs J, Bennink JR, Yewdell JW, Coligan JE, Firat H, Lemonnier F, Ehrlich R.** Human cytomegalovirus protein US2 interferes with the expression of human HFE, a nonclassical class I major histocompatibility complex molecule that regulates iron homeostasis. *J Virol.* 2001; 75: 10557–62.
24. **Drakesmith H, Chen N, Ledermann H, Screaton G, Townsend A, Xu XN.** HIV-1 Nef down-regulates the hemochromatosis protein HFE, manipulating cellular iron homeostasis. *Proc Natl Acad Sci USA.* 2005; 102: 11017–22.
25. **Vahdati-Ben Arieh S, Laham N, Schechter C, Yewdell JW, Coligan JE, Ehrlich R.** A single viral protein HCMV US2 affects antigen presentation and intracellular iron homeostasis by degradation of classical HLA class I and HFE molecules. *Blood.* 2003; 101: 2858–64.
26. **Paulsson K, Wang P.** Chaperones and folding of MHC class I molecules in the endoplasmic reticulum. *Biochim Biophys Acta.* 2003; 1641: 1–12.

27. **Bouvier M.** Accessory proteins and the assembly of human class I MHC molecules: a molecular and structural perspective. *Mol Immunol.* 2003; 39: 697–706.
28. **Dick TP.** Assembly of MHC class I peptide complexes from the perspective of disulfide bond formation. *Cell Mol Life Sci.* 2004; 61: 547–56.
29. **Cresswell P, Bangia N, Dick T, Diedrich G.** The nature of the MHC class I peptide loading complex. *Immunol Rev.* 1999; 172: 21–8.
30. **Neefjes JJ, Momburg F, Hammerling GJ.** Selective and ATP-dependent translocation of peptides by the MHC-encoded transporter. *Science.* 1993; 261: 769–71.
31. **Androlewicz MJ, Anderson KS, Cresswell P.** Evidence that transporters associated with antigen processing translocate a major histocompatibility complex class I-binding peptide into the endoplasmic reticulum in an ATP-dependent manner. *Proc Natl Acad Sci USA.* 1993; 90: 9130–4.
32. **Elliott T, Williams A.** The optimization of peptide cargo bound to MHC class I molecules by the peptide-loading complex. *Immunol Rev.* 2005; 207: 89–99.
33. **Howarth M, Williams A, Tolstrup AB, Elliott T.** Tapasin enhances MHC class I peptide presentation according to peptide half-life. *Proc Natl Acad Sci USA.* 2004; 101: 11737–42.
34. **Zarling AL, Luckey CJ, Marto JA, White FM, Brame CJ, Evans AM, Lehner PJ, Cresswell P, Shabanowitz J, Hunt DF, Engelhard VH.** Tapasin is a facilitator, not an editor, of class I MHC peptide binding. *J Immunol.* 2003; 171: 5287–95.
35. **Park B, Lee S, Kim E, Cho K, Riddell SR, Cho S, Ahn K.** Redox regulation facilitates optimal peptide selection by MHC class I during antigen processing. *Cell.* 2006; 127: 369–82.
36. **Pentcheva T, Edidin M.** Clustering of peptide-loaded MHC class I molecules for endoplasmic reticulum export imaged by fluorescence resonance energy transfer. *J Immunol.* 2001; 166: 6625–32.
37. **Spiliotis ET, Manley H, Osorio M, Zuniga MC, Edidin M.** Selective export of MHC class I molecules from the ER after their dissociation from TAP. *Immunity.* 2000; 13: 841–51.
38. **Goldberg AL, Dice JF.** Intracellular protein degradation in mammalian and bacterial cells. *Annu Rev Biochem.* 1974; 43: 835–69.
39. **Yewdell JW, Anton LC, Bennink JR.** Defective ribosomal products (DRiPs): a major source of antigenic peptides for MHC class I molecules? *J Immunol.* 1996; 157: 1823–6.
40. **Reits EA, Vos JC, Gromme M, Neefjes J.** The major substrates for TAP *in vivo* are derived from newly synthesized proteins. *Nature.* 2000; 404: 774–8.
41. **Schubert U, Anton LC, Gibbs J, Norbury CC, Yewdell JW, Bennink JR.** Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature.* 2000; 404: 770–4.
42. **Qian SB, Reits E, Neefjes J, Deslich JM, Bennink JR, Yewdell JW.** Tight linkage between translation and MHC class I peptide ligand generation implies specialized antigen processing for defective ribosomal products. *J Immunol.* 2006; 177: 227–33.
43. **Yewdell JW, Reits E, Neefjes J.** Making sense of mass destruction: quantitating MHC class I antigen presentation. *Nat Rev Immunol.* 2003; 3: 952–61.
44. **Lybarger L, Wang X, Harris M, Hansen TH.** Viral immune evasion molecules attack the ER peptide-loading complex and exploit ER-associated degradation pathways. *Curr Opin Immunol.* 2005; 17: 71–8.
45. **Ambagala AP, Solheim JC, Srikumaran S.** Viral interference with MHC class I antigen presentation pathway: the battle continues. *Vet Immunol Immunopathol.* 2005; 107: 1–15.
46. **de Almeida SF, Carvalho IF, Cardoso CS, Cordeiro JV, Azevedo JE, Neefjes J, de Sousa M.** HFE cross-talks with the MHC class I antigen presentation pathway. *Blood.* 2005; 106: 971–7.
47. **Freitas AA, Rocha B.** Population biology of lymphocytes: the flight for survival. *Annu Rev Immunol.* 2000; 18: 83–111.
48. **Tanchot C, Lemonnier FA, Perarnau B, Freitas AA, Rocha B.** Differential requirements for survival and proliferation of CD8 naive or memory T cells. *Science.* 1997; 276: 2057–62.
49. **Akolkar PN, Gulwani-Akolkar B, Pergolizzi R, Bigler RD, Silver J.** Influence of HLA genes on T cell receptor V segment frequencies and expression levels in peripheral blood lymphocytes. *J Immunol.* 1993; 150: 2761–73.
50. **Gulwani-Akolkar B, Shi B, Akolkar PN, Ito K, Bias WB, Silver J.** Do HLA genes play a prominent role in determining T cell receptor V alpha segment usage in humans? *J Immunol.* 1995; 154: 3843–51.
51. **Biassoni R, Cantoni C, Pende D, Sivori S, Parolini S, Vitale M, Bottino C, Moretta A.** Human natural killer cell receptors and co-receptors. *Immunol Rev.* 2001; 181: 203–14.
52. **Cardoso C, Porto G, Lacerda R, Resende D, Rodrigues P, Bravo F, Oliveira JC, Justica B, de Sousa M.** T-cell receptor repertoire in hereditary hemochromatosis: a study of 32 hemochromatosis patients and 274 healthy subjects. *Hum Immunol.* 2001; 62: 488–99.
53. **Oberg L, Johansson S, Michaelsson J, Tomasello E, Vivier E, Karre K, Hoglund P.** Loss or mismatch of MHC class I is sufficient to trigger NK cell-mediated rejection of resting lymphocytes *in vivo* - role of

KARAP/DAP12-dependent and -independent pathways. *Eur J Immunol.* 2004; 34: 1646–53.

54. **Pascolo S, Ginhoux F, Laham N, Walter S, Schoor O, Probst J, Rohrlich P, Obermayr F, Fisch P, Danos O, Ehrlich R, Lemonnier FA, Rammensee HG.** The non-classical HLA class I molecule HFE does not influence the NK-like activity contained in fresh human PBMCs and does not interact with NK cells. *Int Immunol.* 2005; 17: 117–22.
55. **Rohrlich PS, Fazilleau N, Ginhoux F, Firat H, Michel F, Cochet M, Laham N, Roth MP, Pascolo S, Nato F, Coppin H, Charneau P, Danos O, Acuto O, Ehrlich R, Kanellopoulos J, Lemonnier FA.** Direct recognition by alphabeta cytolytic T cells of Hfe, a MHC class Ib molecule without antigen-presenting function. *Proc Natl Acad Sci USA.* 2005; 102: 12855–60.
56. **Ten Elshof AE, Brittenham GM, Chorney KA, Page MJ, Gerhard G, Cable EE, Chorney MJ.** Gamma delta intraepithelial lymphocytes drive tumor necrosis factor-alpha responsiveness to intestinal iron challenge: relevance to hemochromatosis. *Immunol Rev.* 1999; 167: 223–32.
57. **Sanchez LM, Chirino AJ, Bjorkman P.** Crystal structure of human ZAG, a fat-depleting factor related to MHC molecules. *Science.* 1999; 283: 1914–9.
58. **Bing C, Bao Y, Jenkins J, Sanders P, Manieri M, Cinti S, Tisdale MJ, Trayhurn P.** Zinc-alpha2-glycoprotein, a lipid mobilizing factor, is expressed in adipocytes and is up-regulated in mice with cancer cachexia. *Proc Natl Acad Sci USA.* 2004; 101: 2500–5.
59. **Kennedy MW, Heikema AP, Cooper A, Bjorkman PJ, Sanchez LM.** Hydrophobic ligand binding by Zn-alpha 2-glycoprotein, a soluble fat-depleting factor related to major histocompatibility complex proteins. *J Biol Chem.* 2001; 276: 35008–13.
60. **Braud VM, Allan DS, McMichael AJ.** Functions of nonclassical MHC and non-MHC-encoded class I molecules. *Curr Opin Immunol.* 1999; 11: 100–8.
61. **Ma Y, Hendershot LM.** The role of the unfolded protein response in tumour development: friend or foe? *Nat Rev Cancer.* 2004; 4: 966–77.
62. **Wu J, Kaufman RJ.** From acute ER stress to physiological roles of the Unfolded Protein Response. *Cell death and differentiation.* 2006; 13: 374–84.
63. **Schroder M, Kaufman RJ.** The Mammalian Unfolded Protein Response. *Annu Rev Biochem.* 2005; 74: 739–89.
64. **de Almeida SF, Fleming JV, Azevedo JE, Carmo-Fonseca M, de Sousa M.** Stimulation of an unfolded protein response impairs MHC class I expression. *J Immunol.* 2007; 178: 3612–9.
65. **Sato M, Sato K, Nakano A.** Evidence for the intimate relationship between vesicle budding from the ER and the unfolded protein response. *Biochem Biophys Res Commun.* 2002; 296: 560–7.
66. **Nyfeler B, Nufer O, Matsui T, Mori K, Hauri HP.** The cargo receptor ERGIC-53 is a target of the unfolded protein response. *Biochem Biophys Res Commun.* 2003; 304: 599–604.
67. **Lewis JW, Elliott T.** Evidence for successive peptide binding and quality control stages during MHC class I assembly. *Curr Biol.* 1998; 8: 717–20.
68. **Harding HP, Zhang Y, Ron D.** Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature.* 1999; 397: 271–4.
69. **Su HL, Liao CL, Lin YL.** Japanese encephalitis virus infection initiates endoplasmic reticulum stress and an unfolded protein response. *J Virol.* 2002; 76: 4162–71.
70. **Tardif KD, Mori K, Siddiqui A.** Hepatitis C virus subgenomic replicons induce endoplasmic reticulum stress activating an intracellular signaling pathway. *J Virol.* 2002; 76: 7453–9.
71. **Liu N, Kuang X, Kim HT, Stoica G, Qiang W, Scofield VL, Wong PK.** Possible involvement of both endoplasmic reticulum- and mitochondria-dependent pathways in MoMuLV-ts1-induced apoptosis in astrocytes. *J Neurovirol.* 2004; 10: 189–98.
72. **Tirosh B, Iwakoshi NN, Lilley BN, Lee AH, Glimcher LH, Ploegh HL.** Human cytomegalovirus protein US11 provokes an unfolded protein response that may facilitate the degradation of class I major histocompatibility complex products. *J Virol.* 2005; 79: 2768–79.
73. **Hegde NR, Chevalier MS, Wisner TW, Denton MC, Shire K, Frappier L, Johnson DC.** The role of BiP in endoplasmic reticulum-associated degradation of major histocompatibility complex class I heavy chain induced by cytomegalovirus proteins. *J Biol Chem.* 2006; 281: 20910–9.
74. **Tardif KD, Siddiqui A.** Cell surface expression of major histocompatibility complex class I molecules is reduced in hepatitis C virus subgenomic replicon-expressing cells. *J Virol.* 2003; 77: 11644–50.
75. **Cardoso EM, Duarte MA, Ribeiro E, Rodrigues P, Hultcrantz R, Sampaio P, Ehrlich R, Carvalho J, Fraga J, de Sousa M.** A study of some hepatic immunological markers, iron load and virus genotype in chronic hepatitis C. *J Hepatol.* 2004; 41: 319–26.
76. **Welch WJ, Brown CR.** Influence of molecular and chemical chaperones on protein folding. *Cell Stress Chaperones.* 1996; 1: 109–15.
77. **Rubenstein RC, Egan ME, Zeitlin PL.** *In vitro* pharmacologic restoration of CFTR-mediated chloride transport with sodium 4-phenylbutyrate in cystic fibrosis epithelial cells containing delta F508-CFTR. *J Clin Invest.* 1997; 100: 2457–65.

78. **Burrows JA, Willis LK, Perlmutter DH.** Chemical chaperones mediate increased secretion of mutant alpha 1-antitrypsin (alpha 1-AT) Z: A potential pharmacological strategy for prevention of liver injury and emphysema in alpha 1-AT deficiency. *Proc Natl Acad Sci USA.* 2000; 97: 1796–801.
79. **Xie Q, Khaoustov VI, Chung CC, Sohn J, Krishnan B, Lewis DE, Yoffe B.** Effect of tauroursodeoxycholic acid on endoplasmic reticulum stress-induced caspase-12 activation. *Hepatology.* 2002; 36: 592–601.
80. **Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, Gorgun CZ, Hotamisligil GS.** Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science.* 2006; 313: 1137–40.
81. **Poupon RE, Bonnand AM, Chretien Y, Poupon R.** Ten-year survival in ursodeoxycholic acid-treated patients with primary biliary cirrhosis. The UDCA-PBC Study Group. *Hepatology.* 1999; 29: 1668–71.
82. **Kaplan MM, Gershwin ME.** Primary biliary cirrhosis. *N Engl J Med.* 2005; 353: 1261–73.
83. **Collins AF, Pearson HA, Giardina P, McDonagh KT, Brusilow SW, Dover GJ.** Oral sodium phenylbutyrate therapy in homozygous beta thalassemia: a clinical trial. *Blood.* 1995; 85: 43–9.
84. **Lawless MW, Mankan AK, White M, O'Dwyer MJ, Norris S.** Expression of hereditary hemochromatosis C282Y HFE protein in HEK293 cells activates specific endoplasmic reticulum stress responses. *BMC Cell Biology.* 2007; 8: 30.
85. **Pinto JP, Ramos P, de Almeida SF, Oliveira S, Breda L, Michalak M, Porto G, Rivella S, de Sousa M.** Protective role of calreticulin in HFE Hemochromatosis. *Free Radic Biol Med.* 2007; 44: 99–108.