Divergent effects of α1-antitrypsin on the regulation of iron metabolism in human erythroleukaemic (K562) and myelomonocytic (THP-1) cells

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The acute-phase protein α1-antitrypsin (α1-AT) has been shown to inhibit the binding of transferrin to its cell-surface receptor. Here we demonstrate that in human erythroleukaemic cells (K562) α1-AT enhances the binding affinity of iron-regulatory protein (IRP), the central regulator of cellular iron metabolism, to iron-responsive elements. Activation of IRP by α1-AT is associated with a marked increase in transferrin receptor (trf-rec) mRNA levels in K562 and enhanced cell-surface expression of transferrin-binding sites, whereas ferritin production is decreased, although ferritin mRNA levels remain unchanged. In agreement with the well-established mechanism of cellular iron regulation, α1-AT seems to modulate trf-rec and ferritin expression primarily post-transcriptionally/translationally by influencing IRP activity. In contrast, α1-AT produces only minor changes in IRP activity, and subsequently in trf-rec expression and ferritin synthesis in THP-1 cells. Moreover the effects of α1-AT on iron homeostasis in K562 cannot be overcome by the addition of iron salts, whereas concomitant treatment of THP-1 with iron and α1-AT results in the same metabolic changes as the addition of iron alone. Because α1-AT blocks transferrin binding on K562 as well as on THP-1 cells, it is suggested, on the basis of the results presented here, (1) that erythroid and monocytic cells might differ in their dependence on transferrin-mediated iron supply and (2) that THP-1 might be able to acquire iron by a transferrin-independent iron uptake system. α1-AT might therefore be involved in the diversion of iron traffic between various cellular compartments under inflammatory conditions.

INTRODUCTION

α1-Antitrypsin (α1-AT) is an acute-phase protein that is mainly produced and released in excess by hepatocytes but also by monocytes/macrophages in response to injurious stimuli, such as infection, trauma or neoplasm [1]. α1-AT, a 56 kDa glycoprotein, is a member of the serpin family of protease inhibitors and a very potent inhibitor of neutrophil elastase, thus preventing tissue damage during an inflammatory process [1–3]. Furthermore α1-AT forms a covalently stabilized complex with human neutrophil elastase. This complex acts as a chemoreactant for human neutrophils [4] and mediates increases in the expression of the α1-AT gene in human monocytes and macrophages [5]. We have demonstrated previously that α1-AT binds to transferrin receptors (trf-recs) on human placental membranes and on the surface of human erythroleukaemic cells (K562), thus inhibiting the interaction of iron-loaded transferrin with its specific receptor as well as the internalization of the transferrin–trf-rec complex [6,7]. α1-AT-induced blockage of transferrin binding to its specific receptor should decrease iron uptake into erythroid cells, which might be one reason for growth inhibition by α1-AT [7]. Interestingly, prolonged exposure to α1-AT resulted in an increased expression of trf-rec on such cells [7]. We have suggested that this might originate from the endeavour of the cell to overcome iron depletion owing to the α1-AT-induced inhibition of transferrin-mediated iron uptake. Therefore we investigated the putative effects of α1-AT on the regulation of cellular iron traffic.

The maintenance of cellular iron homeostasis is largely exerted post-transcriptionally via the interaction of cytoplasmatic proteins, called iron-regulatory protein (IRP)-1 and IRP-2, with RNA stem–loop structures, called iron-responsive elements (IREs) (reviewed in [8–10]). The interaction of IRPs with IREs within the 5′ untranslated region of ferritin mRNA and erythroid 5-aminolaevulinate synthase mRNA, as is evident during iron deprivation states, oxidative stress or nitric oxide formation, results in the repression of translation for these proteins. In contrast, high-affinity binding of IRP to IREs within the 3′ untranslated region of trf-rec mRNA increases the stability of this mRNA, thus promoting trf-rec expression and iron uptake into cells [8–10]. In contrast, an enhanced intracellular availability of low-molecular-mass iron results in the deactivation of the IRE-binding function of IRP-1 via conformational changes in the protein's iron/sulphur cluster, thus reversing the translational inhibition of the expression of ferritin mRNA and erythroid 5-aminolaevulinate synthase mRNA, whereas trf-rec stability is decreased, which therefore favours iron storage (ferritin) and iron consumption (haem biosynthesis) [8–10].

In this study we show that α1-AT differently influences cellular iron regulation via IRPs and as a consequence trf-rec and ferritin expression in human erythroleukaemic (K562) and human myelomonocytic cells (THP-1).

MATERIALS AND METHODS

Cell culture techniques

The human monocytic cell line THP-1 and the human erythroleukaemic cell line K562 were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). Cells were grown in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated foetal calf serum, 100 i.u./ml penicillin and 0.1 mg/ml streptomycin at 37 °C in humidified air containing 5% CO_2. Cells were seeded at a density of 5×10^5/ml of medium, and 50 μM ferric nitrate nonahydrate, 100 μM desferrioxamine (both from Sigma, Munich, Germany) and/or 50 μM human α1-AT (from C.R.T.S., Lille, France) were added. After 24 or 72 h of incubation at 37 °C cells were harvested, washed twice with PBS and subjected to further procedures as indicated below.

Abbreviations used: α1-AT, α1-antitrypsin; IRE, iron-responsive element; IRP, iron-regulatory protein; trf-rec, transferrin receptor.

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α1-AT used for our experiments was characterized by means of immunoelectrophoresis and isoelectric focusing as described previously [6]. To test whether α1-AT was free, protease-complexed [4,5] or proteolytically modified, we also performed SDS/PAGE on α1-AT alone and on the proteolytically modified proteins formed on incubation of α1-AT with human neutrophil elastase (Calbiochem, La Jolla, CA, U.S.A.; specific activity greater than 20 units/mg protein). Figure 1 shows that the α1-AT that we used formed a single band at 56 kDa. In contrast, incubation of this protein with human leukocyte elastase formed the previously described complex at approx. 68 kDa [4,11]. These and previous [6] experiments provide clear evidence that the α1-AT used in this and previous [6,7] studies was a highly purified protein that was neither proteolytically modified nor in a complex with a protease such as human neutrophil elastase. Furthermore transferrin-binding studies in various tissues and in the presence of proteinase inhibitors gave further credibility to the notion that α1-AT rather than a protease-complexed or proteolytically modified form of this acute-phase protein is responsible for the competitive interaction with trf-rec ([6,7], and I. Graziadei, C. Egger, D. Niederwieser, J. R. Patsch and W. Vogel, unpublished work).

Generation of a ^{32}P-labelled IRE probe by transcription *in vitro* and by gel retardation assay

K562 and THP-1 cells were grown in RPMI plus additives, and treated as described above. After harvesting and washing, detergent extracts of cells were prepared as described previously [12]. A ^{32}P-labelled IRE probe was transcribed *in vitro* by T7 polymerase and purified by gel electrophoresis [15% (w/v) acrylamide/bisacrylamide (20:1) plus 6 M urea) followed by probe elution, phenol/chloroform extraction and ethanol precipitation as described [13]. Analysis of RNA–protein complexes was performed by non-denaturing gel electrophoresis and subsequent autoradiography as described [14].

RNA extraction and Northern blot analysis

Cells were stimulated as described above. The preparation of total RNA and Northern hybridization were then performed exactly as detailed elsewhere [15]. Probes were labelled with [α-^{32}P]dCTP (DuPont–New England Nuclear, Boston, MA, U.S.A.) with the oligoprimer procedure [16].

Transferrin-binding studies

These investigations were performed as detailed elsewhere [17,18] with some modifications. Briefly, cells were incubated for 24 or 72 h at 37 °C under 5% CO_2, in RPMI containing 10% foetal calf serum and desferrioxamine (100 μM), iron nitrate (50 μM), α1-AT (50 μM) or no further additives. After this, cells were harvested, washed twice in incubation buffer (RPMI containing 0.1% BSA/10 mM Hepes), and approx. 2×10^6 cells/ml were incubated with 93–186 pM diferric ^{125}I-labelled transferrin at 4 °C in a final volume of 0.25 ml. After incubation for 120 min the reaction was stopped by addition of 2 ml of ice-cold PBS; after consecutive washing steps, the cell-associated ^{125}I activity was determined after correction for non-specific binding, which was estimated after the addition of 10 μM unlabelled diferric transferrin.

Determination of intracellular ferritin

Cells (10^6) were stimulated with the various additives described above, and after 24 or 72 h detergent extracts of cells were prepared [12]. The intracellular ferritin concentration was determined by a commercially available ELISA from DRG Diagnostica (Marburg, Germany) and related to the intracellular protein content (ng of ferritin/mg of protein).

Protein determination

The protein concentration of cell lysates was estimated by the method of Bradford [19] with the protein dye reagent from Bio Rad (Richmond, CA, U.S.A.) and BSA as a standard.

Statistical analysis

Calculation of statistical significance was performed with Student's t test. Only P values below 0.05 were considered significant.

RESULTS

Different regulation of IRP activity by α1-AT in human erythroleukaemic and monocytic cells

Because of our previous observations demonstrating an increased surface expression of trf-rec on K562 cells after prolonged exposure to α1-AT [6,7] we were now interested to elucidate the background of this phenomenon. Because cellular iron regulation is largely exerted post-transcriptionally via IRE/IRP interaction [8–10] we first investigated whether α1-AT could influence the IRE-binding activity of IRP. Figure 1 shows a gel retardation assay of detergent cell extracts from K562 cells. One RNA–protein complex is found in untreated cells (control), corresponding to human IRP-1. As previously described [20], treatment of cells with iron (50 μM) decreases the IRE-binding

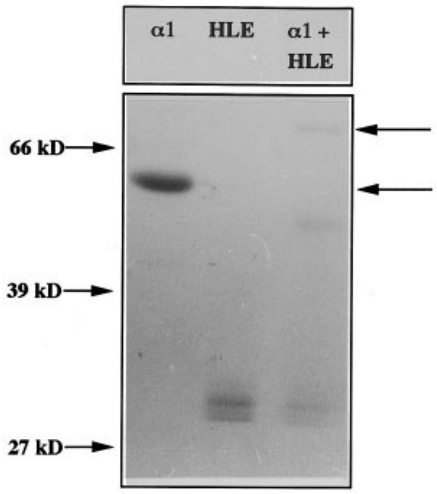

Figure 1 SDS/PAGE of α1-AT

α1-AT (α1; 5 μg) or human neutrophil (leukocyte) elastase (HLE) or a reaction mixture containing α1-AT and HLE in equimolar concentrations were incubated for 30 min at 37 °C. PMSF was then added to each sample to a concentration of 2 mM and the incubation was continued for another 10 min at 37 °C. After this, samples were analysed by SDS/PAGE [15% (w/v) gel] under reducing conditions followed by staining with Coomassie Brilliant Blue. The positions of molecular mass markers are indicated at the left. α1-AT formed a single complex at approx. 56 kDa (lane 1; indicated also by the lower arrow on the right). HLE formed two complexes between 28 and 30 kDa (lane 2). When α1-AT was incubated with HLE the 56 kDa band disappeared but a new band at approx. 68 kDa was observed corresponding to the previously described α1-AT-protease complex (lane 3; indicated by the upper arrow on the right). Abbreviation: kD, kDa.

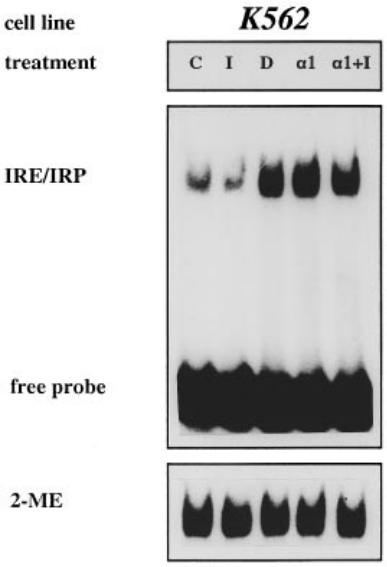

Figure 2 Effects of α1-AT on IRE-binding activity of IRP in K562 cells

K562 cells were treated for 72 h with 50 μM $Fe(NO_3)_3$ (I), 100 μM desferrioxamine (D), 50 μM α1-AT (α1) or were untreated (control, C). Detergent extracts (20 μg) of cells were assayed for IRE-binding activity of IRP by means of a gel-shift assay in the absence (upper panel) or presence (lower panel) of 2% (v/v) 2-mercaptoethanol (2-ME). The IRE–IRP complex and the unbound IRE probe (free probe) are shown in the upper panel, whereas in the lower panel only RNA–protein complexes are pictured. Results from one of five similar experiments are shown.

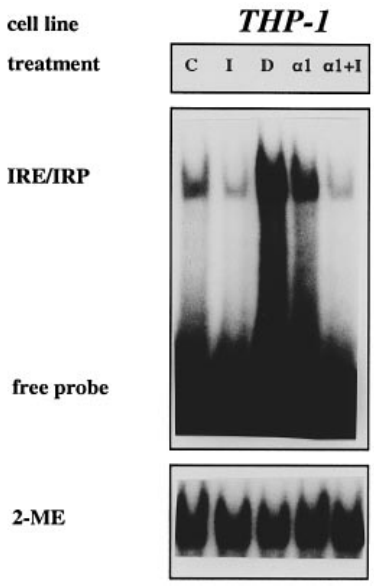

Figure 3 IRE-binding by IRP in THP-1 cells treated with α1-AT

THP-1 cells were treated with various additives and gel retardation assay was performed exactly as described in the legend to Figure 2. Results from one of four representative experiments are shown.

function of IRP whereas the withdrawal of iron on addition of the intracellular iron chelator desferrioxamine (100 μM) clearly enhances IRP activity compared with control cells (Figure 2). Treatment of K562 cells with 50 μM α1-AT for 72 h profoundly increased the IRE-binding activity of IRP compared with the control cells and, surprisingly, IRP activity remained high even on concomitant supplementation with 50 μM iron (Figure 2). All these observed effects on IRP-1 seem to occur post-translationally because the IRE-binding activity of IRP-1 can be fully recovered *in vitro* by treatment with 2% (v/v) 2-mercaptoethanol [12] (Figure 2, lower panel).

To investigate whether these observations might also hold true in other cell types, we next studied the effects of perturbations in α1-AT and iron on IRP activity in the human myelomonocytic cell line THP-1, which has been widely used as a model for exploring the biochemical mechanisms of macrophage-like cells. As in K562 only one RNA–protein complex is detectable in THP-1 cells by means of gel-shift assay corresponding to IRP-1 (Figure 3). Moreover the regulatory effects of perturbations in iron on the IRE-binding activity of IRP-1 were the same as observed in K562, meaning that the addition of iron decreases IRP activity whereas it is enhanced on treatment with desferrioxamine (Figure 3). Interestingly, in comparison with the control, treatment with 50 μM α1-AT resulted only in a moderate increase in the IRE-binding activity of IRP in THP-1 cells (Figure 3; compare lanes 1 and 4), and concomitant treatment of cells with α1-AT and iron decreased IRP activity to levels comparable to that observed after treatment with iron alone (Figure 3; compare lanes 2 and 5). As with K562 cells, alterations of IRP-1 activity seem to occur post-translationally, because the IRE-binding activity of IRP could be fully activated by the treatment of extracts with 2% 2-mercaptoethanol (Figure 3, lower panel).

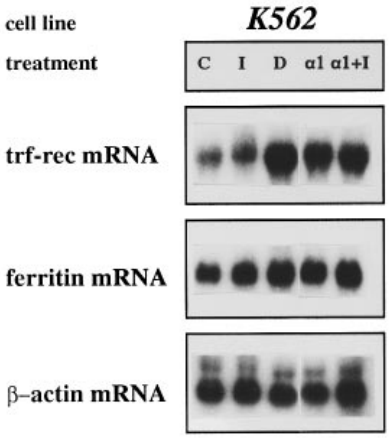

Figure 4 Trf-rec and ferritin mRNA concentrations in response to treatment of K562 cells with α1-AT

K562 cells were treated for 72 h with 50 μM $Fe(NO_3)_3$ (I), 100 μM desferrioxamine (D) or 50 μM α1-AT (α1), or were untreated (control, C). Then total RNA was extracted and 10 μg of this was subjected to Northern blotting with a human trf-rec cDNA, H-ferritin cDNA or a rat glyceraldehyde-3-phosphate dehydrogenase cDNA probe. Results from one of three similar experiments are shown.

Trf-rec mRNA levels and transferrin binding in K562 and THP-1 cells after exposure to α1-AT

Because stimulation of the IRE-binding activity of IRP during iron-deficiency states increases trf-rec mRNA expression via post-transcriptional mRNA stabilization [21,22], we investigated whether modulation of IRP activity by α1-AT in K562 and THP-1 cells would affect trf-rec mRNA levels in a similar fashion. Figure 4 shows a Northern blot obtained from K562 cells treated with the various additives described above. trf-rec mRNA was

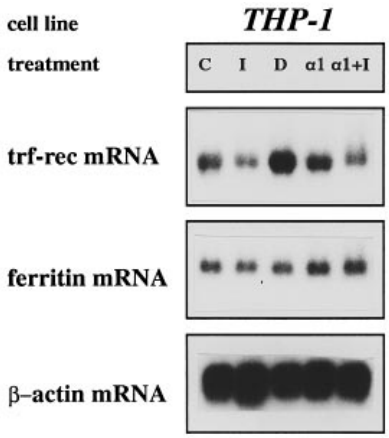

Figure 5 Trf-rec and ferritin mRNA concentrations in THP-1 cells

THP-1 cells were treated with various additives, and Northern blotting was performed as described in the legend to Figure 4. Results from one of three similar experiments are shown.

Table 1 Transferrin receptor expression on the surface of K562 and THP-1 cells

After treatment of cells for 72 h with the indicated stimuli, the determination of cell-surface expression of trf-rec on K562 and THP-1 cells was performed by saturation with ^{125}I-transferrin at 4 °C, after correction for non-specific binding as detailed in the Materials and methods section. Results are expressed as means ± S.D. for three independent experiments. Significance of differences compared with the control (no additions) were calculated by Student's *t* test: * $P < 0.01$; † $P > 0.05$, not significant. Abbreviation: DFO, desferrioxamine.

	$10^4 \times$ Transferrin-binding sites per cell	
Addition	K562	THP-1
None	9.3 ± 0.2	2.51 ± 0.24
Iron	8.4 ± 1.2†	0.81 ± 0.09*
DFO	29.2 ± 3.7*	9.31 ± 1.56*
α1-AT	17.2 ± 2.5*	3.34 ± 0.56†
α1-AT + iron	15.9 ± 2.5*	0.94 ± 0.04*

detectable in control cells, and treatment of cells with desferrioxamine clearly increased trf-rec mRNA levels (Figure 4, compare lanes 1 and 3). Treatment of K562 cells with iron resulted in no obvious effect on trf-rec mRNA expression compared with the control. This phenomenon of diminished iron response has consistently been observed before [23–25] and has been attributed to relative differences in cellular iron status related to growth rate and density of cells as well as to differences in the iron content of the media. However, stimulation of K562 cells with α1-AT, a condition that up-regulates IRP activity (Figure 2), enhanced trf-rec mRNA levels compared with control cells, and trf-rec mRNA concentrations remained high even on concomitant treatment with α1-AT and iron (Figure 4).

In THP-1 cells trf-rec mRNA concentrations were decreased after treatment with iron, and increased after supplementation of desferrioxamine, compared with untreated control cells (Figure 5, compare lanes 1–3), in agreement with the metabolic changes observed in K562 [20] (Figure 4). In contrast, treatment of THP-1 cells with α1-AT caused only a moderate increase of trf-rec mRNA concentrations compared with the control (Figure 5, lane 4), and – similarly to regulation of IRP – concomitant supplementation of iron and α1-AT to THP-1 cells decreased trf-rec mRNA levels to an extent comparable to that observed in iron-loaded cells (Figure 5, compare lanes 2 and 5). These responses in K562 and THP-1 cells are specific because none of the treatments caused significant changes in β-actin mRNA, which was used as an internal control (Figures 4 and 5, bottom panels).

As a consequence of the results obtained so far, we investigated the effects of iron, desferrioxamine and/or α1-AT on the expression of surface trf-rec on K562 and THP-1 cells, determined by saturation experiments performed at 4 °C with pre-incubated cells. As outlined in Table 1, the number of trf-recs was decreased, compared with the control, on treatment of cells with iron and increased on supplementation with desferrioxamine. This was true for K562 and THP-1 cells (Table 1). When K562 cells were treated with α1-AT, transferrin binding was approximately doubled compared with the control. Addition of α1-AT and iron slightly decreased transferrin binding compared with cells treated with α1-AT alone, but transferrin binding in such cells was still significantly higher than in control cells (Table 1).

In contrast, α1-AT only slightly enhanced the maximum density of transferrin-binding sites on THP-1 cells but this increase was completely abolished when the cells were additionally loaded with iron (Table 1).

In accordance with a well-established mechanism [21,22] and with the results presented here, it seems reasonable that alterations in the cell-surface expression of trf-rec are due to post-transcriptional regulation of trf-rec mRNA expression via the modulation of IRE-binding activity of IRP by the various additives. Interestingly, alterations of IRP activity seem to account quantitatively for differences in trf-rec mRNA concentrations and in the number of surface trf-recs.

Ferritin expression is differently modulated in K562 and THP-1 cells exposed to α1-AT

Because modulations of IRP activity were also shown to affect ferritin expression via a translational mechanism [8–10], we investigated whether this would also be the case for IRP regulation by α1-AT. As is evident from Figure 4 (middle lane), ferritin mRNA levels were not significantly altered after treatment of K562 cells with iron, desferrioxamine and/or α1-AT compared with untreated control cells. On the whole the same is also true for THP-1 cells although ferritin mRNA levels seemed to be increased marginally in response to treatment with α1-AT (Figure 5).

Treatment of both K562 and THP-1 cells with iron increased intracellular ferritin levels compared with untreated cells, whereas they were decreased after addition of desferrioxamine (Table 2), which is due to the translational regulation of ferritin expression as previously shown [8–10,23–28].

In comparison with the control, treatment of K562 with α1-AT decreased the cellular ferritin content, which remained low even after additional supplementation with iron (Table 2). In contrast, in THP-1 cells ferritin concentrations were not significantly decreased, compared with the control, after treatment with α1-AT. Moreover, concomitant addition of α1-AT and iron clearly enhanced intracellular ferritin concentration to levels comparable to those observed after stimulation of THP-1 cells with iron alone (Table 2).

Taken together, these findings provide clear evidence that α1-AT regulates ferritin protein expression primarily translationally via the modulation of IRP activity.

Table 2 Intracellular ferritin concentrations in K562 and THP-1 cells

Cells were treated for 72 h with the indicated stimuli, and after preparation of detergent extracts of cells intracellular ferritin concentrations were determined by a commercially available ELISA and related to the intracellular protein content (ng of ferritin/mg of protein). Results are expressed as means ± S.D. for three independent experiments performed in duplicate. Significance of differences compared with the control (no additions) was calculated by Student's *t* test: * $P < 0.01$; ** $P > 0.05$; † $P > 0.05$, not significant. Abbreviation: DFO, desferrioxamine.

	Intracellular ferritin (ng/mg of protein)	
Addition	K562	THP-1
None	259 ± 22	133 ± 14
Iron	329 ± 31**	410 ± 43*
DFO	136 ± 26*	67 ± 12*
α1-AT	152 ± 16*	126 ± 18†
α1-AT + iron	157 ± 34*	482 ± 26*

In general, the regulatory effects of the various additives on all aspects of iron metabolism described above were the same after 24 or 72 h of stimulation, but the relative differences between various treatments were more pronounced after 72 h of incubation.

DISCUSSION

The results presented demonstrate that exposure of K562 cells to the acute-phase protein α1-AT results in metabolic changes in iron homeostasis. Treatment of K562 cells with α1-AT stimulates the IRE-binding function of IRP, which is then followed by an increase in trf-rec mRNA levels and an enhanced expression of transferrin-binding sites on K562 cells. According to the well-established mechanism of post-transcriptional regulation of iron metabolism [8–10,21,22], this should be based on an increase in mRNA stability due to high-affinity binding of IRP to IREs within the 3′ untranslated region of trf-rec mRNA. This protects trf-rec mRNA from degradation by an RNase that has not yet been fully characterized, which consequently enhances trf-rec expression. At the same time, interaction of activated IRP with an IRE-motif within the 5′ untranslated region of ferritin mRNA causes translational repression of ferritin expression via inhibition of the attachment of 40 S ribosomal subunit to this mRNA [8–10,23–29].

However, the capacity of α1-AT to modulate the post-transcriptional regulation of iron was clearly different between THP-1 and K562 cells. On the one hand the effects of α1-AT on iron homeostasis in K562 cells were comparable to those observed during iron-deprivation states (desferrioxamine treatment) and these metabolic changes could not be abolished by additional supplementation with iron. On the other hand, treatment of human monocytic cells (THP-1) with α1-AT did not cause highly significant differences in cellular iron regulation compared with untreated cells, and further addition of iron resulted in similar changes in the post-transcriptional regulation of iron metabolism to those described for the treatment of cells with iron alone [8–10,20–28].

These variations in response to α1-AT, in differently modulating IRP-1 activity and subsequently trf-rec and ferritin expression in the two cell lines, cannot be related to the fact that α1-AT interacts with trf-rec on K562 but not on THP-1 cells. It has been demonstrated that (1) α1-AT interferes with trf-rec on K562 [7] as well as on THP-1 cells (I. Graziadei, C. Egger, D. Niederwieser, J. R. Patsch and W. Vogel, unpublished work) and (2) α1-AT completely inhibits the binding of transferrin to its surface receptor and the internalization of the transferrin/trf-rec complex in both cell lines ([7], and I. Graziadei, C. Egger, D. Niederwieser, J. R. Patsch and W. Vogel, unpublished work), which indicates that an alternative mechanism must be responsible for these divergent effects of α1-AT on intracellular iron regulation in these two cell lines.

There is increasing evidence that various cell types might differ in their mechanisms of iron uptake and their dependence on trf-rec-mediated iron ingestion [30,31]. Uptake of iron from transferrin via trf-rec-mediated endocytosis has been well described in erythroid cells such as K562 [32–35]. Alternatively it has been shown that various cell lines, such as HeLa cells, are able to acquire iron very effectively via a membrane-based transport system that has as yet not been fully characterized, regulated in a different manner from the transferrin-mediated pathway [36,37]. The results presented here fit nicely into this scheme of different dependence of cells on trf-rec-mediated iron uptake and would also provide an explanation for the divergent effect of α1-AT on post-transcriptional regulation of iron in K562 and THP-1 cells. As mentioned above, exposure of K562 or THP-1 cells to α1-AT blocks the interaction of iron-loaded transferrin with cell-surface trf-rec ([7], and I. Graziadei, C. Egger, D. Niederwieser, J. R. Patsch and W. Vogel, unpublished work). This should then result in decreased transferrin-mediated iron uptake into cells. Because K562 cells are believed to be primarily dependent on transferrin/trf-rec-mediated iron uptake [32–35], treatment with α1-AT will decrease intracellular iron availability, which might be one reason for the decreased proliferation rate of these cells in the presence of α1-AT [7]. As described in detail elsewhere [8–10], the limitation of metabolically available iron to the cell results in a conformational change in the central iron/sulphur cluster of IRP-1, thus stimulating the IRE-binding function of IRP-1. High-affinity interaction of IREs with IRP then results in the down-regulation of iron storage via the repression of ferritin translation, whereas iron uptake is promoted via the increased expression of trf-rec due to stabilization of its mRNA [8–10,20–28]. This is consistent with the results presented here, demonstrating that α1-AT enhances IRP activity in K562 and subsequently decreases ferritin synthesis, whereas trf-rec expression is increased. In contrast, α1-AT had no significant effects on post-transcriptional regulation of iron via IRP in THP-1 cells. On the basis of the results of Kaplan and co-workers [36,37] and observations made in activated human monocytes [38] it seems reasonable that THP-1 monocytic cells are also able to acquire iron via a transferrin-independent iron uptake system. This would be consistent with our finding that the blocking of trf-rec-mediated iron uptake on addition of α1-AT is not sufficient to cause iron deprivation in THP-1 cells. Therefore, as shown here, IRP activity does not change significantly on treatment of THP-1 with α1-AT, and as a consequence post-transcriptional regulation of the expression of ferritin mRNA and trf-rec mRNA is not obviously altered compared with control cells. From our results it seems reasonable that THP-1 cells are able to compensate for the elimination of trf-rec-mediated iron uptake very effectively, because concomitant administration of iron and α1-AT resulted in the same metabolic changes as administration of iron alone, which provides further evidence for the existence of a transferrin-independent iron uptake system in monocytic cells such as THP-1.

In summary, the observation of contrasting effects of α1-AT on the post-transcriptional regulation of iron between erythroid and monocytic cells suggests different mechanisms of iron uptake by these compartments. However, one can only speculate about

the importance of this divergence, for example in an inflammatory response. It is well established that under such conditions increased amounts of acute-phase proteins, such as α1-AT, are produced in the liver and released into the circulation [1]. Divergent effects of α1-AT towards iron uptake by erythroid and monocytic cells might contribute to the diversion of iron traffic underlying the anaemia of chronic disease [39–41]. According to the results presented here and published previously [7], exposure of erythroid cells to α1-AT would result in a diminished transferrin-mediated uptake of iron into these cells. In contrast, iron uptake into monocytes/macrophages by the proposed transferrin-independent mechanism should not be substantially affected by α1-AT, and the supply of iron to monocytes/macrophages might even be favoured owing to a decreased iron consumption by the erythron in the presence of α1-AT. Iron plays an important role in the cytotoxic effector function of macrophages, e.g. via its catalytic function for the formation of toxic hydroxyl radicals by the 'Haber–Weiss' reaction [42,43]. Therefore the efficient uptake of iron by macrophages under inflammatory conditions would contribute to an increase in the antimicrobial and antitumour effector potential of macrophages [42,43]. In addition, the increased uptake and storage of iron by activated macrophages might also strengthen host defence, e.g. via stimulation of cell-mediated immune effector functions as described previously [40,41].

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