Effect of aluminium on iron uptake and transferrin-receptor expression by human erythroleukaemia K562 cells

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Incubation of human erythroleukaemia K562 cells with Al-transferrin inhibited iron uptake from ^{59}Fe -transferrin by about 80 %. The inhibition was greater than that produced by a similar quantity of Fe-transferrin. Preincubation of cells for 6 h with either Al-transferrin or Fe-transferrin diminished the number of surface transferrin receptors by about 40 % compared with cells preincubated with apo-transferrin. Al-transferrin did not compete significantly with Fe-transferrin for transferrin receptors and, when cells were preincubated for 15 min instead of 6 h, the inhibitory effect of Al-transferrin on receptor expression was lost. Both forms of transferrin also decreased the level of transferrin receptor mRNA by about 50 %, suggesting a common regulatory mechanism. Aluminium citrate had no effect on iron uptake or transferrin-receptor expression. AlCl_3 also had no effect on transferrin-receptor expression, but at high concentration it caused an increase in iron uptake by an unknown, possibly non-specific, mechanism. Neither Al-transferrin nor AlCl_3 caused a significant change in cell proliferation. It is proposed that aluminium, when bound to transferrin, inhibits iron uptake partly by down-regulating transferrin-receptor expression and partly by interfering with intracellular release of iron from transferrin.

INTRODUCTION

Aluminium overload is recognized as a cause of disorders in patients on haemodialysis and in whom it may cause bone defects (osteomalacia), anaemia and, in some cases, encephalopathy (Savory *et al.*, 1985). There is also evidence [reviewed by Ganrot (1986)] implicating aluminium in the pathogenesis of Alzheimer's disease. The mechanisms by which aluminium gives rise to these pathological conditions is unknown, and indeed little is known about how aluminium is transported in the body and incorporated into cells. It has been established that aluminium can bind to the specific metal-binding sites of the iron-transport protein transferrin, and most if not all plasma aluminium is bound to transferrin (Trapp, 1983). This suggests that aluminium may enter cells in the same way as iron, i.e. via the transferrin–transferrin-receptor system of receptor-mediated endocytosis, and it has been reported that Al-transferrin interacts with the transferrin receptor in a similar way to Fe-transferrin (Morris *et al.*, 1987; Feng *et al.* 1988). Thus aluminium, if bound to transferrin, could interfere with uptake of iron and induce anaemia and proliferative abnormalities.

To investigate this problem, we have studied the effect of different forms of aluminium on iron uptake, transferrin-receptor expression and proliferation of the erythroleukaemic cell line K562, in which the mechanisms of iron uptake have been well established (Klausner, 1988).

EXPERIMENTAL

Cell culture

The K562 cell line was routinely cultured in RPMI 1640 medium (Flow laboratories, Rickmansworth, Herts., U.K.) containing 100 i.u. of penicillin/ml + 100 μg of streptomycin/ml (Flow Laboratories) and 10 % fetal-calf serum (Flow Laboratories). Before use the cells were spun down, washed, and resuspended in serum-free medium (see below).

Aluminium content of reagents and apparatus

The aluminium content of all reagents used was checked by atomic-absorption spectroscopy and found to be < 2 ng/ml. However, the transferrin used (obtained from Behringwerke, Hounslow, Middx., U.K.) was found to contain 60 ng of aluminium/mg of transferrin. This was partially removed by dialysis against 0.1 M-sodium citrate, pH 5.1, followed by two changes of deionized distilled water, after which the aluminium content had decreased to 30 ng of aluminium/mg of transferrin. This represents approx. 6 % of the transferrin metal-binding capacity.

Plasticware was used throughout, and aluminium contamination of each batch checked by soaking overnight in 1 % HNO_3 followed by atomic-absorption spectroscopy of the acid solution. All reagents were prepared in a laminar-flow cabinet to prevent contamination with aluminium from the environment. The final aluminium content of the tissue culture medium was between 1 and 2 ng/ml.

Binding of iron to transferrin

This was carried out by adding iron nitrilotriacetate (FeNTA) to apotransferrin dissolved in phosphate-buffered saline (PBS)/0.1 % NaHCO_3 to give the required saturation with iron, essentially as described by Graham & Bates (1976). The solution was then left overnight to allow binding to occur. Where necessary, [^{59}Fe]ferric citrate (Amersham International; sp. radioactivity 10 $\mu\text{Ci}/\mu\text{g}$) was included to give a final activity of 5 $\mu\text{Ci}/\text{mg}$ of transferrin and 30 % iron saturation.

Preparation of Al-transferrin

A 25 μM solution of aluminium citrate in 0.05 M-Tris/HCl buffer, pH 7.4, was added to a solution of apo-transferrin (10 mg/ml) in Tris buffer containing 1 mg of NaHCO_3 /ml at a ratio of 10.4 μl per mg of transferrin. The solution was left at 4 °C for 1 h and then dialysed for 24 h against Tris buffer to

Abbreviations used: FeNTA, iron nitrilotriacetate; PBS, phosphate-buffered saline (0.01 M-phosphate/0.14 M-NaCl, pH 7.2).

remove unbound aluminium. The A_{280} was measured and the amount of transferrin was determined by using an $A_{280}^{1\text{mg/ml}}$ value of 1.28, calculated from the data of Trapp (1983). Binding of aluminium to transferrin was confirmed by measuring A_{240} against apo-transferrin, which gives a value of 0.22 for a 0.9 mg/ml solution (Trapp, 1983).

AlCl₃ and aluminium citrate

AlCl₃ was obtained from BDH (Poole, Dorset, U.K.). Aluminium citrate was prepared from AlCl₃ and a 5 molar excess of trisodium citrate. Both were made up in Tris/HCl buffer.

Effect of aluminium on iron uptake by K562 cells

The effect of Al-transferrin on iron incorporation into K562 cells was tested by incubating 3×10^6 cells with 10 μg of ^{59}Fe -transferrin (30% satd.)/ml in the presence of 40 μg of Al-transferrin/ml. Controls were incubated with apo-transferrin or 80%-iron-saturated transferrin. In some experiments the saturation of ^{59}Fe -transferrin was raised to 90% by the subsequent addition of the appropriate amount of unlabelled FeNTA. To test the effect of AlCl₃ and aluminium citrate, appropriate dilutions of the aluminium salt and ^{59}Fe -transferrin at 50 μg /ml were added to similar cultures. This procedure ensured that in all cases the final concentration of transferrin was 50 μg /ml. Control cultures contained no aluminium. In both sets of experiments the final volume was 3 ml, made up with serum-free medium containing 1 mg of human serum albumin (Sigma, Poole, Dorset, U.K.)/ml. After 6 h incubation at 37 °C, the cells were spun down, washed three times with Hanks balanced salts solution and the ^{59}Fe radioactivities of the supernatants, washes and cells were determined in a LKB Compugamma γ -radiation counter (LKB, Croydon, Surrey, U.K.). Before the final centrifugation the cell suspension was transferred to a new tube to avoid errors due to non-specific binding of ^{59}Fe to the original tube. All cultures were adjusted to contain the same quantity of Tris/HCl buffer.

Labelling of transferrin with ^{125}I

Fully saturated transferrin was prepared as described above and iodinated by the chloramine-T method as described previously (Oria *et al.*, 1988), the reaction time being decreased to 20 s to avoid denaturation. Unbound ^{125}I was removed by passage through a prepacked disposable Sephadex G-25M column (Pharmacia, Uppsala, Sweden).

Effect of aluminium on transferrin-receptor expression by K562 cells

Cultures of K562 cells were set up as described above, except that unlabelled 30%-iron-saturated transferrin was added instead of ^{59}Fe -transferrin. (This was added to ensure that conditions were identical to those used in the iron uptake experiments.) The cells were incubated for 6 h as described above, and were then spun down, washed twice in ice-cold PBS containing 0.2% BSA (BSA-PBS; BSA was from Sigma) and resuspended at 2×10^7 cells/ml in BSA-PBS. Aliquots containing 5×10^5 cells were then added to the wells of a 96-well round-bottomed microtitre plate which had previously been coated to reduce binding of ^{125}I -transferrin by incubation for 1 h at 37 °C with 0.25% gelatin in PBS containing 1% BSA and then washed twice in ice-cold BSA-PBS. An appropriate dilution of ^{125}I -transferrin in BSA-PBS was added to each well. Non-specific binding was determined by adding a 200-fold excess of unlabelled transferrin. All subsequent steps were carried out at 0 °C. The plate was incubated for 1 h in an ice bath and the cells were then harvested on to a glass-fibre mat using a cell harvester (Ilacon, Tonbridge, Kent, U.K.). Each well was washed eight times with

ice-cold BSA-PBS, and the ^{125}I activity of the filters was determined. The number and affinity of transferrin receptors were calculated by the method of Scatchard (1949), and where necessary the significance of the inhibitory effect of Al-transferrin or Fe-transferrin determined using a one-sample *t* test.

In separate experiments, the time of incubation with Al-transferrin and controls was changed to 15 min or 24 h. In these experiments the cells were preincubated for 1 h in serum-free medium before incubation with Al-transferrin to prevent possible interference by serum components.

Inhibition of binding of Fe-transferrin by Al-transferrin was determined in a similar way, except that the initial culture was performed in medium containing 50 μg of apo-transferrin/ml to maximize transferrin-receptor expression. Aliquots of the cell suspension were then incubated for 1 h with 10 μg of ^{125}I -transferrin/ml in the presence of various concentrations of Al-transferrin, Fe-transferrin or apo-transferrin, and binding of ^{125}I -transferrin was assessed as above.

Estimation of transferrin-receptor mRNA

K562 cells were incubated for 6 h with apo-transferrin, Al-transferrin or Fe-transferrin for 6 h as described above. Full details of subsequent methods are available from J.H.B. on request. Briefly, total cell RNA was extracted using RNazol (Cinna/Biotech, Friendswood, TX, U.S.A.) and samples were slot-blotted on to nitrocellulose membranes. After prehybridization these were probed with random primer-labelled transferrin-receptor cDNA pTR32 (Schneider *et al.*, 1984; kindly provided by Dr. J. Williams, Imperial Cancer Research Fund, London W.C.1., U.K.) and with an oligonucleotide probe to ribosomal RNA (synthesized in the Beatson Institute for Cancer Research), which was used to normalize variations in loading. Northern blots were carried out with both probes and showed that, in both cases, hybridization was specific for RNA of the correct size. Autoradiographs were scanned on a GS300 densitometer (Hoefer, Newcastle-under-Lyme, Staffs., U.K.).

Cell proliferation

K562 cells at an initial density of 2×10^6 /ml were grown overnight in serum-free medium containing apo-transferrin (50 μg /ml), in the presence of Al-transferrin (100 μg /ml), or AlCl₃ (6.8 or 0.068 μg of aluminium/ml). The cells were then pulsed for 4 h with 20 μCi of [^3H]thymidine (Amersham International; sp. radioactivity 5 Ci/mmol)/ml, harvested, and the extent of thymidine incorporation compared with that shown by controls cultured in medium without aluminium compounds.

RESULTS

Effect of Al-transferrin on iron uptake

Uptake of ^{59}Fe from ^{59}Fe -transferrin was diminished by at least 80% in the presence of Al-transferrin, compared with uptake in the presence of a similar quantity of apo-transferrin (Table 1). The effect of Al-transferrin was about twice that produced by excess 80%-iron-saturated transferrin. When the saturation of the ^{59}Fe -transferrin was increased from 30% (the normal physiological saturation in man, at which most transferrin would be in the monoferric form) to 90% (at which most transferrin would be diferric) the inhibitory effect of Al-transferrin on iron uptake was proportionally similar, even though, as expected, absolute uptake increased. This makes it unlikely that the effect seen with transferrin at 30% saturation was due to displacement of monoferric transferrin by di-Al-transferrin, even though molecules with both metal-binding sites

Table 1. Effect of Al-transferrin on Fe uptake by K562 cells

K562 cells (10^6 /ml) were incubated for 6 h with $10 \mu\text{g}$ of ^{59}Fe -transferrin/ml, either 30% or 90% saturated, in the presence of $40 \mu\text{g}$ of apo-transferrin, Al-transferrin (fully saturated) or Fe-transferrin (80% saturated)/ml in serum-free RPMI 1640 containing 1 mg of human serum albumin/ml. The results are means $\pm$ S.D. for three experiments. Values in parenthesis represent percentage inhibition of uptake compared with cells incubated with apo-transferrin.

Culture containing:	Iron uptake (ng/ 10^6 cells) from ^{59}Fe -transferrin at an iron saturation of:	
	30%	90%
Apo-transferrin	1.75 ± 0.25	5.03 ± 0.59
Fe-transferrin	1.09 ± 0.19 (38)	3.26 ± 0.32 (35)
Al-transferrin	0.33 ± 0.04 (81)	0.91 ± 0.04 (82)

Table 2. Effect of Al-transferrin on transferrin-receptor expression by K562 cells

K562 cells (10^6 /ml) were incubated for 6 h with $10 \mu\text{g}$ of Fe-transferrin (30% saturated)/ml in the presence of $40 \mu\text{g}$ of apotransferrin, Al-transferrin (fully saturated) or Fe-transferrin (80% saturated)/ml in serum-free RPMI 1640 containing 1 mg of human serum albumin/ml. The number of cell-surface transferrin-binding sites was then determined by incubation for 1 h at 0°C with ^{125}I -transferrin, non-specific binding being determined by addition of a 200-fold excess of unlabelled transferrin. The results are means $\pm$ S.D. for four experiments and show the percentage decrease in the number of transferrin-binding sites compared with cells incubated in the presence of apotransferrin (mean value 3.2×10^5 binding sites/cell). The reduction was statistically significant in both cases ($P < 0.05$).

Culture containing:	Relative no. of transferrin-binding sites per cell
Apo-transferrin	100
Fe-transferrin	37 ± 19
Al-transferrin	42 ± 16

occupied are preferentially bound by transferrin receptors (Young *et al.*, 1984). Hence displacement of labelled transferrin appears not to be the main mechanism involved.

Effect of Al-transferrin on transferrin-receptor expression

To determine whether the inhibition of iron incorporation caused by Al-transferrin was due to a decrease in expression of cell-surface transferrin receptors, the effect of Al-transferrin on binding of Fe-transferrin to the cells was examined. Compared with cells incubated with apo-transferrin, Al-transferrin caused a 42% decrease in the number of surface binding sites (Table 2), which was similar to the effect of Fe-transferrin. Thus the decrease in iron uptake caused by Al-transferrin appears to be partially, though not entirely, due to inhibition of transferrin-receptor expression.

To investigate further the way in which Al-transferrin affected transferrin-receptor expression, cells were exposed to Al-transferrin, apotransferrin or Fe-transferrin for a much shorter (15 min) or much longer (24 h) time. It was found (Table 3) that, when incubated for only 15 min in the presence of Al-transferrin, no decrease in the number of surface transferrin receptors was observed compared with cells incubated with apo-transferrin.

Table 3. Effect of Al-transferrin on transferrin-receptor expression by K562 cells after 15 min or 24 h incubation

Details are as for Table 2, except that cells were preincubated for 1 h in serum-free medium before incubation with Al-transferrin or controls. Results are from one representative experiment and show the number of surface binding sites per cell, with (in parentheses) the degree of inhibition compared with cells incubated with apo-transferrin.

Culture containing:	Period of incubation ...	$10^{-5} \times$ No. of transferrin-binding sites	
		15 min	24 h
Apo-transferrin		0.70	0.63
Fe-transferrin		0.41 (42)	0.45 (29)
Al-transferrin		0.70 (1)	0.41 (35)

Fig. 1. Effect of Al-transferrin on binding of Fe-transferrin to K562 cells

K562 cells were incubated for 6 h in the presence of $50 \mu\text{g}$ of apotransferrin/ml. Binding of ^{125}I -transferrin ($10 \mu\text{g}/\text{ml}$) was then assessed in the presence of 0–500 $\mu\text{g}/\text{ml}$ of unlabelled Al-transferrin (●), Fe-transferrin (■) or apo-transferrin (▲), and compared with the results from controls containing no unlabelled transferrin.

Fe-transferrin still caused a decrease, though this was probably due to competition from pre-bound Fe-transferrin, as it is unlikely that over such a short period any intracellular accumulation of iron could have occurred to down-regulate transferrin-receptor expression. By contrast, the results obtained after 24 h of incubation were similar to those after 6 h shown in Table 2, i.e. both Al-transferrin and Fe-transferrin caused a decrease in surface transferrin-receptor number.

Effect of Al-transferrin on binding of Fe-transferrin

Al-transferrin had only a modest inhibitory effect on the binding of ^{125}I -labelled Fe-transferrin to K562 cells, a 50-fold excess producing less than 50% inhibition (Fig. 1). This was slightly greater than the effect of apo-transferrin, but much less than the effect of Fe-transferrin, indicating that Al-transferrin is unlikely to compete significantly with Fe-transferrin for cell-surface receptors.

Effect of Al-transferrin on transferrin-receptor mRNA levels

The level of transferrin-receptor mRNA in K562 cells that had been incubated for 6 h with Al-transferrin was reduced by 54%

(after correction for loading from the ribosomal RNA hybridization) compared with the level in cells incubated with apo-transferrin (Fig. 2). Cells incubated with Fe-transferrin showed a similar reduction (50 %).

Effect of aluminium citrate and AlCl_3

To determine whether the inhibitory effect on iron uptake and transferrin-receptor expression caused by Al-transferrin required the aluminium to be bound to transferrin, the effect of AlCl_3 and aluminium citrate was investigated (Table 4). Aluminium citrate at aluminium concentrations of either 0.068 or 6.8 $\mu\text{g}/\text{ml}$ had no effect, the lower concentration being equivalent to the amount of aluminium present in Al-transferrin in the previous experiments. AlCl_3 at the lower concentration also had no effect on iron uptake, but at the higher concentration AlCl_3 unexpectedly produced an increase in ^{59}Fe incorporation. However, neither aluminium citrate nor AlCl_3 had any marked effect on transferrin-receptor expression. Thus the stimulation of iron uptake by AlCl_3 appears not to be due to increased transferrin-receptor expression.

Effect of aluminium on cell proliferation

Incubation of K562 cells with either AlCl_3 or Al-transferrin for 24 h had no effect on $[^3\text{H}]$ thymidine incorporation (Table 5).

Fig. 2. mRNA levels in K562 cells

K562 cells were incubated for 6 h in the presence of Fe-transferrin (Fe), apotransferrin (apo) or Al-transferrin (Al) for 6 h. Total mRNA was extracted, slot-blotted and probed with the pTR32 transferrin-receptor cDNA (TfR) and with a synthetic oligonucleotide probe to ribosomal RNA (rRNA).

DISCUSSION

The work reported here shows clearly that aluminium, when bound to transferrin, inhibits uptake of transferrin-bound iron by the erythroleukaemic K562 cell line. The inhibitory effect of Al-transferrin was greater than that of a comparable amount of Fe-transferrin, indicating that inhibition was not simply due to competition between Al-transferrin and the ^{59}Fe -transferrin (Table 1). Furthermore, the degree of inhibition was not affected by increasing the iron saturation of the ^{59}Fe -transferrin from 30 to 90 %.

The inhibitory effect of Al-transferrin on iron uptake was probably due in part to an inhibition of transferrin-receptor expression, as incubation of K562 cells with Al-transferrin for 6 h caused a 42 % reduction in the number of surface binding sites for transferrin (Table 2). However, in this case the effect was comparable with, rather than greater than, the effect of Fe-transferrin. Since transferrin-receptor expression is regulated by levels of metabolically available intracellular iron (Klausner, 1988), it seems likely that aluminium enters this regulatory pool and mimics the effect of iron. Support for this explanation is provided by the experiments showing that the inhibitory effect of Al-transferrin on transferrin-receptor expression is not apparent after only 15 min of incubation (Table 3), which would be too short a time for any significant uptake of aluminium (or iron) to occur. Furthermore, Al-transferrin was found to have only a

Table 5. Effect of aluminium on proliferation of K562 cells

K562 cells ($2 \times 10^6/\text{ml}$) were incubated for 24 h in serum-free medium containing 50 μg of apo-transferrin/ml and either Al-transferrin or AlCl_3 . Proliferation was determined by pulsing with $[^3\text{H}]$ thymidine (20 $\mu\text{Ci}/\text{ml}$) for 4 h. Results are means $\pm$ S.D. ($n = 10$).

Addition	$[^3\text{H}]$ Thymidine uptake as percentage of control
None (control)	100
Al-transferrin (100 $\mu\text{g}/\text{ml}$)	94 $\pm$ 6
AlCl_3	
6.8 μg of Al/ml	98 $\pm$ 7
0.068 μg of Al/ml	98 $\pm$ 4

Table 4. Effect of AlCl_3 and aluminium citrate on Fe uptake and transferrin-receptor expression by K562 cells

To determine iron uptake, K562 cells ($10^6/\text{ml}$) were incubated for 6 h with 50 μg of ^{59}Fe -transferrin (30 % saturated)/ml in the presence of AlCl_3 or aluminium citrate at the stated concentrations in RPMI 1640 containing 1 mg of human serum albumin/ml. To determine the number of surface transferrin receptors, cells were incubated as above, except that ^{59}Fe -transferrin was replaced by unlabelled Fe-transferrin, and the number of surface binding sites determined as described in Table 2. For iron uptake the results are means $\pm$ S.D. for five to eleven experiments with AlCl_3 , or the mean result for duplicate experiments with aluminium citrate in which variation from the mean was $< 3\%$. For transferrin-receptor expression the results are means $\pm$ S.D. for seven experiments. Values in parentheses represent percentage inhibition compared with cells incubated in the absence of aluminium compounds.

Culture containing:	Al concn. ($\mu\text{g}/\text{ml}$)	Iron uptake (ng/ 10^6 cells)	$10^{-5} \times$ No. of transferrin binding sites
No addition	0	11.5 $\pm$ 3.5	1.6 $\pm$ 1.0
AlCl_3	6.8	21.7 $\pm$ 6.6 (–89)	1.8 $\pm$ 0.2 (–12)
	0.068	11.2 $\pm$ 1.1 (3)	1.5 $\pm$ 0.3 (6)
Aluminium citrate	6.8	11.0 (4)	1.8 $\pm$ 0.1 (–12)
	0.068	12.0 (–4)	1.6 $\pm$ 0.1 (0)

minor effect on the binding of Fe-transferrin (Fig. 1), indicating that direct competition for cell-surface receptors between Al-transferrin and Fe-transferrin is not responsible for the inhibitory effect of Al-transferrin. The effect of Al-transferrin and Fe-transferrin after 24 h was similar to the effect after 6 h, suggesting that by 6 h a state of equilibrium had been reached. Abreo *et al.*, (1990) found that prolonged incubation of murine Friend erythroleukaemia cells with Al-transferrin led to increased transferrin-receptor expression and iron uptake, and suggested that this was due to depletion of intracellular iron. This did not occur in the case of K562 cells, perhaps because they had not been stimulated to differentiate and synthesize haemoglobin, which would increase the iron requirement. Also, murine cells may not behave in the same manner as human cells.

Further support for the idea that aluminium, when bound to transferrin, down-regulates transferrin-receptor expression by mimicking the effect of iron is provided by the finding that levels of transferrin receptor mRNA were reduced by similar amounts in cells incubated with either Al-transferrin or Fe-transferrin. Levels of transferrin-receptor mRNA are determined largely by stability, which is enhanced in the absence of iron by the binding of a cytoplasmic protein to a potential stem-loop structure in the 3'-untranslated region of the message (Klausner, 1988). It thus seems possible that aluminium, like iron, can regulate the synthesis and/or binding of this regulatory protein.

Although inhibition of transferrin-receptor expression may be partly responsible for the decrease in iron uptake, it cannot be the only mechanism, as the effect on iron uptake exceeded that of Fe-transferrin, whereas both Al-transferrin and Fe-transferrin had comparable effects on receptor expression. It has been reported that gallium, an element whose chemistry resembles that of aluminium can, when bound to transferrin, inhibit iron uptake, possibly by blocking acidification of the endocytotic vesicle and thus the release of iron from transferrin (Chitambar & Seligman, 1986). Aluminium delivered via transferrin might exert a similar effect. However, Ga-transferrin caused an increase in transferrin-receptor expression (Chitambar *et al.*, 1983), suggesting that intracellular handling of aluminium and gallium is not identical.

In contrast with the inhibitory effect of Al-transferrin on transferrin-receptor expression and iron uptake, no inhibition occurred if aluminium was added in the form of AlCl_3 or aluminium citrate. Previous studies have indicated that iron citrate is handled differently from Fe-transferrin by cells and may not enter normal intracellular metabolic pathways (White & Jacobs, 1978; Brock, 1981; Taylor *et al.*, 1987), and Abreo *et al.*, (1990) have found that aluminium citrate, unlike Al-transferrin, does not inhibit haemoglobin synthesis *in vitro*. Therefore non-transferrin-bound aluminium probably either fails to enter the cell, or does so in a way that does not intersect with uptake and utilization of transferrin-bound iron.

Although aluminium citrate and AlCl_3 were found not to inhibit iron uptake or transferrin-receptor expression, high concentrations of AlCl_3 surprisingly caused an increase in iron uptake, though not of transferrin-receptor expression. The reason for this is unclear, and may represent an experimental artefact, as the effect was much less pronounced in media containing fetal-calf serum (results not shown), in which serum proteins may sequester the aluminium. In contrast the presence of fetal-calf serum did not greatly alter the inhibitory effects of Al-transferrin (results not shown). However, in tissue-culture medium AlCl_3 will probably hydrolyse to $\text{Al}(\text{OH})_3$, which is a well-known immunological adjuvant and can, for example, increase release of interleukin 1 by monocytes (Mannhalter *et al.*, 1985), suggesting increased membrane turnover. Thus a more rapid recycling of transferrin receptors might account for the increased

iron uptake in the presence of AlCl_3 , despite the lack of increased cell-surface-receptor expression.

Neither Al-transferrin nor AlCl_3 affected the rate of proliferation of K562 cells, despite their effects on iron uptake. It may be that, even in the presence of Al-transferrin, the cells still obtain enough iron for proliferation-associated metabolic events. Much of the iron acquired from transferrin by K562 cells *in vitro* is found in ferritin (Mattia *et al.*, 1986), indicating that uptake considerably exceeds the amount required for cell metabolism. Studies on the fate of iron acquired in the presence of Al-transferrin would help to clarify this point, as also would studies on the long-term effect of aluminium. The same explanation could also account for the failure of AlCl_3 to enhance proliferation, although increased proliferation in response to aluminium salts has been reported for other cell lines (Jones *et al.*, 1986).

On the basis of these findings we would propose that Al-transferrin interacts with transferrin receptors and interferes with iron uptake by two mechanisms. The first involves intracellular release of aluminium and accumulation of this metal in the metabolic pool(s) responsible for control of transferrin-receptor expression, thus down-regulating receptor numbers by reducing the stability of transferrin-receptor mRNA in a manner analogous to iron. Although aluminium can bind to transferrin, it probably cannot be incorporated into the iron-binding sites of haem proteins and enzymes in which the metal-binding sites generally have a much more stringent requirement for iron. Furthermore, a claim that aluminium can be taken up by ferritin (Cochran & Chawtur, 1988) has been disputed (Dedman *et al.*, 1989). Thus it is likely that aluminium would accumulate in transit pools. In addition, aluminium may, like gallium, interfere with acidification of endocytotic vesicles, resulting in inhibition of iron release from transferrin. Aluminium itself may still be released, as it is bound to transferrin much less strongly than is iron (Martin *et al.*, 1987) and its release would probably require a smaller decrease of intravesicular pH.

The results presented here thus suggest that some of the clinical effects of aluminium may result from an interference with normal cellular iron uptake. In particular, the results offer a possible explanation of the microcytic anaemia, which is often associated with aluminium overload in renal failure (Parkinson *et al.*, 1981), and the inhibition of *in vitro* erythropoiesis (Mladenovic, 1988). It is also of interest that the accumulation of aluminium in neurons reported in patients with Alzheimer's diseases (Perl & Brody, 1980) correlates with the presence of transferrin receptors (Hill *et al.*, 1985), although other areas of the brain which do not show accumulation of Al are also rich in transferrin receptors. Further studies will therefore be required to determine why aluminium tends to localize in certain tissues, but it may relate to differences in intracellular handling of aluminium.

We thank Dr. D. Halls, Glasgow Royal Infirmary, for analysing aluminium contamination of materials and reagents. M.N. acknowledges receipt of a training grant from the Principado de Asturias and R.O. an EEC Sectoral Grant. This work was supported by the Scottish Hospitals Endowments Research Trust.

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Received 23 February 1990/18 July 1990; accepted 20 July 1990