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Transferrin binding and iron uptake by mouse lymph node cells during transformation in response to Concanavalin A

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Summary. Mouse lymph node cells cultured with Concanavalin A (Con A) in serum-free medium containing ^{59}Fe-transferrin took up ^{59}Fe more rapidly than cells cultured without Con-A. Uptake of iron commenced rapidly and preceded the onset of DNA synthesis in stimulated cells. Total uptake of transferrin during culture was much lower than that of iron, indicating that cells could remove iron from transferrin. The released transferrin appeared to be undegraded. Lymphoblasts bound six times more transferrin per cell than small lymphocytes. Lymphocytes also took up iron from citrate and nitrilotriacetate complexes, and iron so acquired was not readily removed by desferrioxamine, indicating that it was not bound extracellularly.

INTRODUCTION

In the preceding paper (Brock, 1981) it was shown that the transformation of mouse lymphocytes in response to Concanavalin A or lipopolysaccharide in serum-free medium was greatly enhanced by the presence of iron and transferrin. A requirement for transferrin in the transformation of human lymphocytes has also been reported (Phillips & Azari, 1975; Dillner-Centerlind, Hammerström & Perlmann, 1979). The ability of transferrin to donate iron to erythroid precursors by a specific mechanism is now well established (Aisen & Brown, 1977) and it therefore seems likely that transferrin may be required to provide iron in a suitable form to lymphocytes as a result of an increased requirement for this metal during transformation. It has, however, been suggested that transferrin may perform additional functions during lymphocyte transformation, for example supplying zinc (Phillips, 1978) or binding potentially toxic cations (Iscove & Melchers, 1978). In this paper, we report that transformation of mouse lymphocytes in response to Con A is accompanied by uptake of transferrin-bound iron, and that the uptake mechanism shows some of the characteristics of that of erythroid precursors.

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MATERIALS AND METHODS

Transferrins and other iron-binding agents were obtained as described in the preceding paper (Brock, 1981).

Iron and transferrin uptake

Transferrin was labelled with ^{59}Fe using ^{59}Fe-nitrilotriacetate, and with ^{125}I by the Bolton–Hunter method, as described previously (Esparza & Brock,

1980). Citrate and nitrilotriacetate complexes of ^{59}Fe were prepared using molar ratios of 20:1 for citrate to iron and 4:1 for nitrilotriacetate to iron to ensure the formation of low molecular weight rather than hydrated polymeric complexes (Spiro, Bates & Saltman, 1967). Cultures in serum-free medium containing ^{59}Fe (0·1 μCi/ml; specific activity 3–10 mCi/mg) were set up as described in the preceding paper (Brock, 1981) except that in some cases larger cultures (30ml) from which 1 ml aliquots were removed as required were used.

Iron and transferrin uptake were assessed by centrifuging the cells (1200 r.p.m. for 2 min) and washing twice in 2 ml of cold Hanks's solution. To avoid problems of adsorption of isotopes to the culture tubes, cells were transferred to new tubes after suspension in the second wash solution or, alternatively, controls with no cells were set up. Cells and supernatant were counted in an ICN Tracerlab gamma-counter, and simultaneous counting of ^{125}I and ^{59}Fe performed as described previously (Esparza & Brock, 1980). DNA synthesis was determined by uptake of [^{14}C]-thymidine, as described in the preceding paper (Brock, 1981).

Release of ^{125}I-transferrin from lymphocytes

Lymphoblasts from a 30 ml culture of Con A transformed mouse lymphocytes were harvested, washed twice with 10 ml of Hanks's solution and resuspended in 5ml of medium containing 50 μg/ml of ^{125}I-^{59}Fe transferrin (30% iron saturated). After incubation for 4 hr at 37° the cells were spun down, washed twice in ice-cold Hanks's solution and then incubated for a further 1 hr in fresh medium containing unlabelled transferrin (50 μg/ml). The cells were then harvested and washed twice with 10 ml of Hanks's solution. The supernatant was precipitated with an equal volume of 20% (w/v) trichloroacetic acid, and the radioactivity in the supernatant, precipitate and cell pellet determined.

Removal of bound iron by desferrioxamine

Washed lymphoblasts obtained as described above were resuspended in 3 ml of medium containing ^{59}Fe bound to transferrin, nitrilotriacetate or citrate. After incubation for 16 hr the cells were harvested, washed twice in Hanks's solution and transferred to new tubes containing fresh medium with 1mM desferrioxamine. After incubation for 1 hr at either 37° or 0° the cells and supernatant were separated by centrifugation and assayed for ^{59}Fe.

Binding of transferrin by lymphoblasts and small lymphocytes

Washed lymphoblasts, obtained as described above, and small lymphocytes from mouse peripheral lymph nodes (8×10^6 cells in each case) were incubated in 2 ml of serum-free culture medium containing 50 μg/ml of ^{125}I-transferrin (30% iron saturated). The proportion of blasts was determined using Leishmann's stain. After incubation the cells were centrifuged, washed twice in ice-cold Hanks's solution and the amount of ^{125}I-transferrin associated with the cell pellet determined. Cell-free controls were set up to allow for binding of ^{125}I-transferrin to the culture tubes.

RESULTS

Iron uptake from transferrin and thymidine synthesis by transforming lymphocytes

Transforming lymphocytes progressively took up iron from transferrin (Fig. 1). In the absence of Con A, uptake was much lower, especially after the first 24 hr

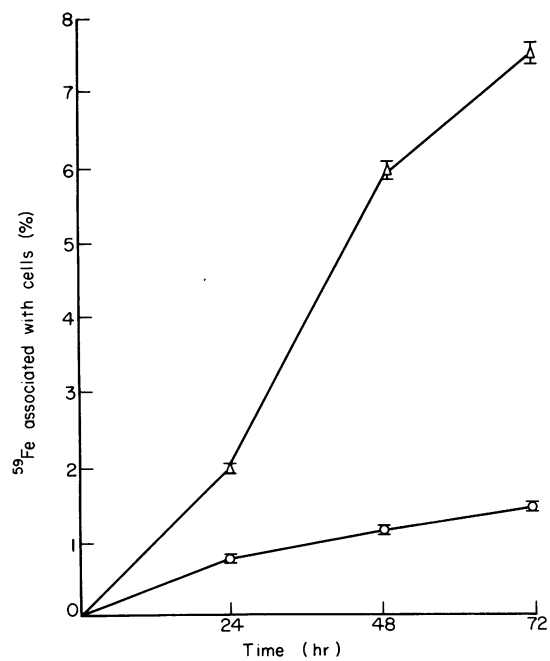

Figure 1. Uptake of ^{59}Fe from 30% saturated transferrin by mouse lymph node cells cultured in serum-free medium in the presence (▵) or absence (○) of Concanavalin A (1 μg). Vertical bars represent 2 standard deviations ($n = 3$).

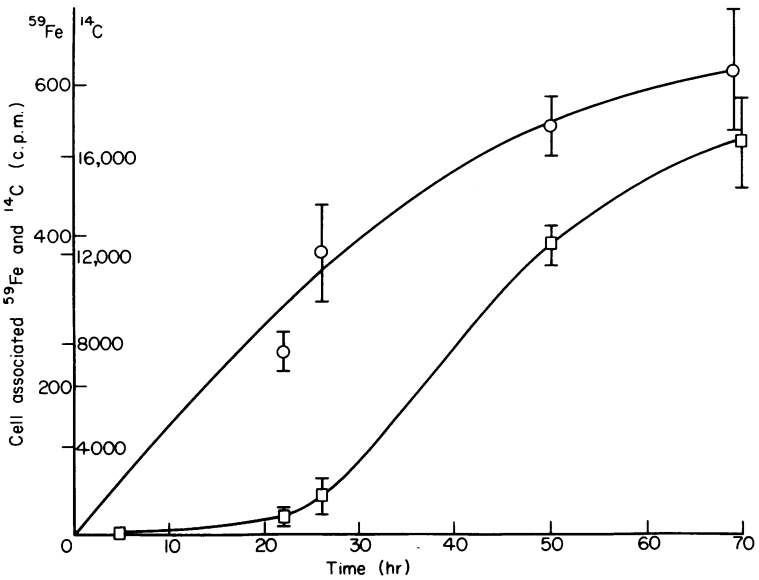

Figure 2. Uptake of ^{59}Fe (□) and [^{14}C]-thymidine (○) by mouse lymph node cells cultured in serum-free medium containing 50 μg/ml of 30% saturated transferrin and 1 μg/ml Concanavalin A. The points on the [^{14}C]-thymidine uptake curve represent the mid-point of 4 hr pulse periods; ^{59}Fe-transferrin was present from zero time. Vertical bars represent 2 standard deviations ($n = 3$).

of incubation, thus indicating an enhanced requirement for iron during transformation. Iron uptake clearly preceded the onset of DNA synthesis, which only became significant after 24 hr of culture (Fig. 2).

Uptake of iron from low molecular weight chelates

A comparison of iron uptake from ^{59}Fe-labelled transferrin, citrate and nitrilotriacetate showed that iron was taken up from the chelates considerably more readily than from transferrin (Table 1), despite the fact that these chelates, unlike transferrin, do not enhance DNA synthesis in response to Con A (Brock, 1981). The cells were however, incapable of taking up desferrioxamine-bound iron.

To determine whether chelate iron was being bound extracellularly to the cell membrane rather than incorporated into the cells, the ability of desferrioxamine to remove iron taken up from chelates or from transferrin was tested. At 37° desferrioxamine removed twice as much iron from the chelate-incubated cells as from the transferrin-incubated cells (Table 2). However, much less iron was removed in all cases by desferrioxamine at 0° suggesting that most iron, whether acquired from chelates or from transferrin was intracellular and not accessible to desferrioxamine unless the latter entered the cell.

Table 1. Uptake by mouse lymph node cells of iron from 30% saturated transferrin and from low molecular weight chelates by mouse lymph node cells cultured in serum-free medium containing Concanavalin A (1 μg/ml)

	Uptake (p mol/10^6 cells)	
Iron compound	3 hr	70 hr
Transferrin	0·4	11·2
Nitrilotriacetate	4·3	46·1
Citrate	3·7	47·8
Ferrioxamine	0·5	0·9

The iron concentration was 0·015 μg/ml in each case. Ferrioxamine-iron uptake was performed in the presence of 1 mM desferrioxamine.

Binding and release of transferrin

Transformation of mouse lymph node cells by Con A in the presence of ^{59}Fe-^{125}I- transferrin revealed that,

while iron was progressively accumulated by the cells the amount of transferrin bound by the cells increased somewhat during the first 24 hr, and thereafter remained fairly constant (Fig.3). The cells were thus clearly removing iron from transferrin. This was confirmed when lymphoblasts were incubated with doubly-labelled transferrin and then 'chased' with cold transferrin. Over half of the labelled transferrin bound by the cells was released, but only 5% of the iron (Table 3). Nearly all the released transferrin was precipitable by 20% trichloroacetic acid, indicating that removal of iron from transferrin by the cells did not involve degradation of the protein.

Table 2. Removal of iron from mouse lymphoblasts by desferrioxamine following incubation with Fe-transferrin, Fe citrate or Fe-nitrilotriacetate

	% Removed by desferrioxamine	
Iron acquired from	37°	4°
Fe-transferrin	18	11
Fe-nitrilotriacetate	38	14
Fe-citrate	40	17

For further details see text.

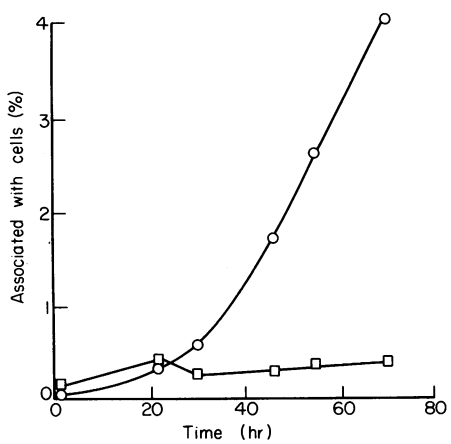

Figure 3. Iron and transferrin binding by mouse lymph node cells incubated in serum-free medium in the presence of Concanavalin A (1μg/ml) and ^{59}Fe, ^{125}I-transferrin (30% iron saturated). (o) ^{59}Fe; (□) ^{125}I. For further details see text.

Binding of transferrin by lymphoblasts and small lymphocytes

To determine whether transferrin-binding by lymphocytes is increased when transformation occurs, the ability of small lymphocytes and Con A-transformed lymphoblasts to bind ^{125}I-labelled transferrin was compared. It was found that on a per cell basis, lymphoblasts bound at least six times as much transferrin as small lymphocytes (Table 4). This increased binding of transferrin must presumably occur early during the proliferative response, as only a modest increase in transferrin binding was observed during a 3-day culture with Con A (see Fig. 3).

Table 3. Distribution of ^{59}Fe and ^{125}I in cell pellet and supernatant fraction after incubation of mouse lymphoblasts with ^{59}Fe, ^{125}I-transferrin followed by a 'chase' of unlabelled transferrin

	Percentage	
	^{125}I	^{59}Fe
Cell pellet	47	95
Supernatant, TCA-insoluble fraction	44	1
Supernatant, TCA-soluble fraction	9	4

For details see text.

Table 4. Binding of transferrin to small lymphocytes and lymphoblasts

	% Blasts	Transferrin binding, μg/10^6 cells
Small lymphocytes	0	0·021
Lymphoblasts	77	0·121

Small lymphocytes were obtained from mouse peripheral lymph nodes, and lymphoblasts by Concanavalin A stimulation of mouse lymph node cells. For further details see text.

DISCUSSION

In this study we have shown that proliferating mouse lymphocytes take up iron from transferrin during culture, the transferrin being released without degradation. In contrast, iron uptake from transferrin by an epithelial cell line was accompanied, by degradation of transferrin (Hemmaplardh & Morgan, 1974).

The iron uptake mechanism in lymphocytes clearly implies the existence of specific cell receptors for transferrin, and these have indeed been recently demonstrated in transforming lymphocytes (Larrick & Cresswell, 1979a,b). The present study has shown that less transferrin is bound by normal than by transforming lymphocytes, in agreement with the finding of others (Larrick & Cresswell, 1979b; Galbraith, Goust, Mercurio & Galbraith, 1980). It thus seems likely that the requirement for transferrin in lymphocyte transformation in serum-free medium, and for proliferation of other cell types (Rudland, Durbin, Clingan & Jiménez de Asua, 1977; Barnes & Sato, 1979; Breitman, Collins & Keene, 1980) is due at least in part to an increased need for iron during transformation.

However, it is necessary to explain the fact that lymphocytes also readily take up iron from nitrilotriacetate and citrate complexes, despite the fact such complexes cannot substitute for transferrin in enhancing DNA synthesis in response to mitogens (Brock, 1981). Also, it was found (Brock, 1981) that fully iron-saturated transferrin is less effective than partially-saturated transferrin, suggesting that additional functions such as supplying zinc or binding toxic cations may also be involved. Low molecular weight iron chelaters would be unable to fulfil either of these functions, thus supporting the idea that while transferrin is primarily involved in supplying iron, this may not be its only function.

White & Jacobs (1978) showed that Chang cells also took up iron more rapidly from citrate or nitrilotriacetate complexes than from transferrin, and suggested that these complexes probably bypassed the specific uptake mechanism needed to acquire transferrin-bound iron. The iron taken up by lymphocytes from these chelates appeared to be mainly intracellular, rather than bound extracellularly. In Chang cells a high proportion of chelate iron, but not iron originating from transferrin, was bound to the cell membrane, but was not easily removed by desferrioxamine (White & Jacobs, 1978). The failure of desferrioxamine to remove appreciable amounts of chelate-acquired iron from lymphocytes at 4° suggests that if chelate iron is indeed largely membrane-bound, binding occurs after entry into the cell, rather than extracellularly. In any case, it must be remembered that iron uptake from chelates is non-physiological, since under normal conditions no low molecular weight iron complexes are present in plasma (Hahn & Ganzoni, 1980). Iron uptake was found to precede DNA synthesis, a result which agrees with the observations of Larrick & Cresswell (1979b) who showed that an increase in binding of transferrin by transforming lymphocytes also preceded DNA synthesis. The results of transferrin binding to lymphocytes and lymphoblasts reported here also point to an early increase in transferrin binding following the addition of mitogen. Taken together, these observations suggest that iron is required in the initial stages of DNA synthesis, and may in particular be involved with production of ribonucleotide reductase, which requires iron (Hoffbrand, Ganeshaguru, Hooton & Tattersall, 1976).

We thus conclude that lymphocyte transformation is accompanied by an increased requirement for iron and that the cells meet this requirement by obtaining iron from transferrin via a specific mechanism. Lymphocytes can, however, also take up chelate-bound iron *in vitro*, and the possibility that transferrin fulfils functions in addition to supplying iron cannot be excluded.

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