Biochimica et Biophysica Acta, 511 (1978) 430—441

BBA 78102

BINDING SITES OF IRON TRANSFERRIN ON RAT RETICULOCYTES

INHIBITION BY SPECIFIC ANTIBODIES

C. VAN DER HEUL [a], M.J. KROOS [b] and H.G. VAN EIJK [b]

Departments of [a] *Internal Medicine I and* [b] *Chemical Pathology, Medical Faculty, Erasmus University, Rotterdam (The Netherlands)*

(Received December 15th, 1977)
(Revised manuscript received April 10th, 1978)

Summary

1. In the process of iron uptake by precursors of the erythrocytes probably more than one membrane component is involved; besides the specific transferrin receptor, another membrane component with a high iron activity after incubation with ^{59}Fe can be isolated.

2. A striking resemblance exists between rat and human reticulocyte components which are involved in the process of iron uptake.

3. Incubation of reticulocytes with F_{ab} fragments of an antibody against the membrane receptor for transferrin causes a concentration-dependent decrease in transferrin binding and iron uptake.

4. The membrane receptor complex isolated is still heterogeneous; analytical ultracentrifugation studies suggest a molecular weight lower than 230 000.

5. Intact immature red cells are necessary for specific binding of transferrin with the receptor followed by iron uptake. This is the only mechanism for iron uptake.

6. Immunofluorescence studies showed that the receptor for transferrin is localized at the outside of the cell membrane.

Introduction

It has been shown that immature red cells are able to take up iron from the plasma iron-binding protein, transferrin, to utilize it for haem synthesis. Also, it is known that the uptake of iron from transferrin is a property which is lost when the immature red cell matures in an erythrocyte [1].

Since Jandl [1] postulated in 1959 that the uptake of iron by precursors of red blood cells is a process which involves a binding of transferrin to, probably,

specific receptors on the cell membrane, many articles have been appeared on this subject [2—17]. Several of these articles describe the conditions and influences (such as incubation time, incubation temperature, pH, degree of saturation of transferrin, synthetic chelating agents, proteolytic enzymes) on which this process is dependent. Although much information has been obtained, the real mechanism by which iron is taken up by immature erythroid cells and transported to the haem-synthesizing mitochondria is not clearly understood.

Almost everybody agrees with the idea that the initial step in this process is the binding of transferrin to specific receptors on the cell membrane; however, the physiological role of these receptors and their composition are unknown.

The opinions about the processes involved in the uptake of iron into the cell, after the binding of transferrin to the receptors on the cell membrane, are contradictory. One opinion [18,19] is that transferrin releases its iron at the receptor site on the cell membrane, after which the iron is transported through the cell membrane to intracellular components and transferrin is released from the cell membrane again.

Some authors [20—22] report that the transferrin · iron complex moves from the receptor or with the receptor into the cell, probably by endocytosis, and releases its iron intracellularly, after which the transferrin molecule is excreted from the cell again.

A few studies have been published which describe the isolation of possible transferrin · receptor complexes on the cell membrane varying in molecular mass from 30 000 to 425 000 [18,19,23—26,35]. One of the first descriptions of the possible transferrin receptor on the cell membrane was published in 1974 by Speyer and Fielding [18,19], who described for human reticulocytes three membrane components which were involved in iron transport into the cell. One of these components, classified as B_2, probably represents the transferrin · receptor complex. The other components, classified as A and B_1, may function as intermediates in the iron transport from the receptor to intracellular components. Results for rabbit reticulocytes are different [25].

Besides our interest in the composition of the receptor for transferrin, we wanted to investigate the specific physiological role of such a receptor during the process of transferrin binding and iron uptake by the cell. We were anxious to know whether blocking of the receptor with its antibody was attended with a decrease in iron uptake, which would emphasize the physiological role of the receptor.

Materials and Methods

All chemicals used were of analytical reagent grade.

Reticulocytes. Reticulocytosis was produced in male Wistar rats by removal of 5 ml of blood by orbital puncture each day for 5 days, with a 3-day interval between the third and fourth specimen, which resulted in a reticulocytosis of 25—65% [28]. The blood was collected into heparinized tubes and the cells were washed with cold phosphate-buffered NaCl solution. After the first washings the buffy coat was removed.

Erythrocytes. Erythrocytes were obtained from normal male Wistar rats.

Bone marrow cells. Bone marrow cells were isolated as described previously [27]. Differential nucleated cell counts of the cell suspensions were carried out on dried cell smears, stained with May-Grünwald-Giemsa solutions.

Transferrin and anti-transferrin. Rat transferrin and antibodies raised against rat transferrin were isolated by affinity chromatography on CNBr-activated Sepharose-4B as has been described previously [30].

Immunoglobulines against ratserum free from transferrin. Serum from which transferrin had been removed by affinity chromatography was injected in rabbits in order to evoke antibodies. The immunoglobulin fraction from this antiserum was isolated by Sephadex G-150 and DEAE-cellulose chromatography [31].

Immunoglobulins against the IgG fraction of rat serum. Antibodies against IgG from rat were raised in rabbits. The immunoglobulin fraction from the antiserum was isolated as described above, using affinity chromatography.

Immunoglobulins against total reticulocyte membrane, against the probable transferrin receptor fraction B_2 and against parts of the membrane different from B_2. Antibodies against the total reticulocyte membrane, against the isolated B_2 fraction and against parts of the membrane different from B_2 were also raised in rabbits. The immunoglobulin fractions of these sera were isolated as described above. All these antibodies were tested by means of an Ouchterlony technique.

Purification of the anti-B_2 immunoglobulins. Anti-transferrin antibodies were removed from antiserum raised to the B_2 fraction (not completely pure) by means of affinity chromatography on a transferrin-Sepharose column.

Splitting the immunoglobulins in F_{ab} and F_c fragments. The purified anti-B_2, the anti-total reticulocyte membrane and the anti 'non B_2'-membrane fragments immunoglobulin fractions were split into F_{ab} and F_c fragments, using the techniques as described by van Eijk [31].

Radioisotope-labelled transferrin. ^{59}Fe was obtained as sterile ferric citrate of specific radioactivity 5—20 Ci/g of Fe and ^{125}I as sodium iodide, carrier-free and free from reducing agents, from the Radiochemical Centre, Amersham, Bucks., U.K. Iron-saturated transferrin was labelled with ^{125}I following the method of Katz [33] using iodine in $Na^{125}I$ solution. Apotransferrin was prepared from iron-saturated transferrin and was labelled with ^{59}Fe as previously described [34].

Incubation experiments. Incubation was carried out in plastic tissue culture dishes at a temperature of 37° C and in an atmosphere of air/CO_2 (95 : 5) 30 min, except where the effect of incubation time and incubation temperature as variables was being investigated. The incubation medium consisted of minimum essential medium with Hanks' salts and HEPES buffer (20 mmol/l) supplemented with foetal bovine serum (10% v/v). With this incubation medium we followed the incubation procedure described by Speyer and Fielding [18,19]. A 50% (v/v) suspension of reticulocytes in Hanks' solution (pH 7.4) was warmed to 37° C and a one-fifth part of its volume of doubly-labelled transferrin solution was added. The final concentration of transferrin protein was 1.4 mg/ml. Under these conditions nonspecific binding of transferrin was avoided [18,19,28]. Immediately after incubation, the tubes were chilled in ice-cold water and 2 vols. of ice-cold 310 ideal milliosmolar sodium phosphate buffer, pH 7.4, was added.

Next the cell suspension was washed five times with 8 vols. of ice-cold 310 ideal mosM buffer. The cell suspension was haemolysed following the method of Dodge [29]; the membranes obtained were solubilized and chromatographed on Sepharose-2B and Sepharose-6B as described by Speyer and Fielding [18, 19]. The experiments in which we used erythrocytes, unsealed reticulocytes ghosts and solubilized reticulocyte membranes were performed in the same manner.

Blocking the receptors for transferrin. Reticulocytes were incubated, during 30 min, with several concentrations of anti-B_2 IgG, anti-total reticulocyte membrane IgG and anti-IgG against the other membrane components. In other experiments the reticulocytes were preincubated with several concentrations of F_{ab} fragments, obtained from anti-B_2 IgG, at 4° C for 30 min.

After washing six times with ice-cold 310 ideal mosM buffer, the cells were incubated with doubly-labelled transferrin as has been described above. Control experiments were performed with F_{ab} fragments from rabbit IgG against rat IgG, against rat serum free of transferrin and against the other membrane components except B_2. The transferrin binding and iron uptake by the reticulocyte was determined by measuring the radioactivity in a Packard autogamma scintillation spectrometer 5120.

Immunofluorescence studies. Bone marrow cells from normal and anaemic Wistar rats were incubated as has been described earlier [27]. After this incubation had been stopped, the cell suspension was incubated with antibodies against rat transferrin or with rabbit antibodies against total rat bone marrow cell membranes at 0° C for 30 min. This incubation was stopped by washing the cell suspension six times with 310 ideal mosM buffer. Finally the cells suspension was incubated with Fluorchrome conjugated goat antiglobulin against rabbit IgG (GAR-FITC).

The percentage of fluorescent cells was determined. Several control experiments were performed with antibodies against rat serum proteins excluding rat transferrin. In these experiments the specific membrane fluorescence was absent.

Purification of the B_2 fraction. In some experiments the nonionic detergent Triton X-100 was removed from the B_2 fraction by means of Bio-beads in a way described by Holloway [36].

Analytical ultracentrifuge experiments. These were performed with a Beckman ultracentrifuge model E. Due to the presence of Triton X-100 the graphs were recorded at 295 nm. At this wavelength Triton X-100 shows hardly any absorption.

Results

Immunofluorescence studies

Immunofluorescence studies of bone-marrow cells, incubated with transferrin and studied with indirect immunofluorescent techniques, showed a distinct membrane fluorescence. After performing some of these experiments, we incubated the bone marrow cell suspension, without preincubation with transferrin, directly with the anti-transferrin antibody. These experiments resulted in a membrane fluorescence of approx. 34% of the cells. The bone marrow con-

TABLE I

FLUORESCENCE OF ERYTHROID CELLS

Experiments: a: + anti rat transferrin antibodies results in agreement between erythroid cell count and percent fluorescence; b: + anti-bone marrow antibodies results in nearly 100% fluorescence; c and d: without antibodies, no fluorescence; e: with antibodies against (rat serum minus transferrin), no fluorescence. +, added; —, absent.

	Experiment	Substances added to bone marrow cells					Observations	
		Anti-rat transferrin IgG	Anti-bone marrow IgG	Anti-IgG against (rat serum minus transferrin)	Hanks' solution	GAR—FITC	Percentage erythroid cells	Percentage fluorescence
Bone marrow from normal rats	a	+	—	—	—	+	36	34
	b	—	+	—	—	+	39	100
	c	—	—	—	+	+	38	2
	d	—	—	—	—	+	36	1
	e	—	—	+	—	+	37	1
Bone marrow from anaemic rats (anaemic by orbital puncture)	a	+	—	—	—	+	53	50
	b	—	+	—	—	+	51	99
	c	—	—	—	+	+	49	3
	d	—	—	—	—	+	50	1
	e	—	—	+	—	+	51	1

tained 36—39% erythroid cells. After inducing anaemia, the fluorescence increased to 50%, while the erythroid cell content increased to 53% (Table I).

These results and the results of control experiments indicate that the fluorescence is specific for the immature red cells and that during the process of iron uptake by immature red cells in vivo transferrin is bound at the outside of the cell membranes. The bone marrow cells were incubated with anti-rat transferrin, anti-bone marrow antibodies, with Hank's solution alone and with the fluorescent dye. Incubations were performed at 4°C for 30 min. If anti-rat transferrin antibodies are added, there is extremely good agreement between the erythroid cell count and the percent of fluorescence, if antibodies to bone marrow cells are added, nearly 100% fluorescence is observed. Without incubation with antibodies no fluorescence is observed.

Incubation experiments

We were able to isolate two radioactive membrane components from rat reticulocytes on Sepharose 2B (Fig. 1). As shown in Fig. 1 the ^{59}Fe and ^{125}I peaks do not coincide, which does suppose that the B component in rat reticulocyte membranes is not homogeneous. Rechromatography of the B peak on Sepharose 6B resulted in three radioactive peaks B_1, B_2 and B_3 (Fig. 2), confirming the heterogeneity of peak B in Fig. 1. B_2 contains a part of the iron activity and almost all iodine activity [18,19,37].

Based on these results we have the opinion that, as in human reticulocytes, B_2 represents the transferrin · receptor complex. B_3 represents in part a probably nonspecific transferrin binding membrane component, because of the fact that this fraction reacts with antibodies against the total reticulocyte membrane, giving rise to a ^{125}I- and ^{59}Fe-labelled precipitate. It was shown that the last part of the B_3 peak represents unbound transferrin, as it does not react with antibodies against the total reticulocyte membrane components, but it does with anti-transferrin.

In order to investigate the necessity of an intact receptor on immature red

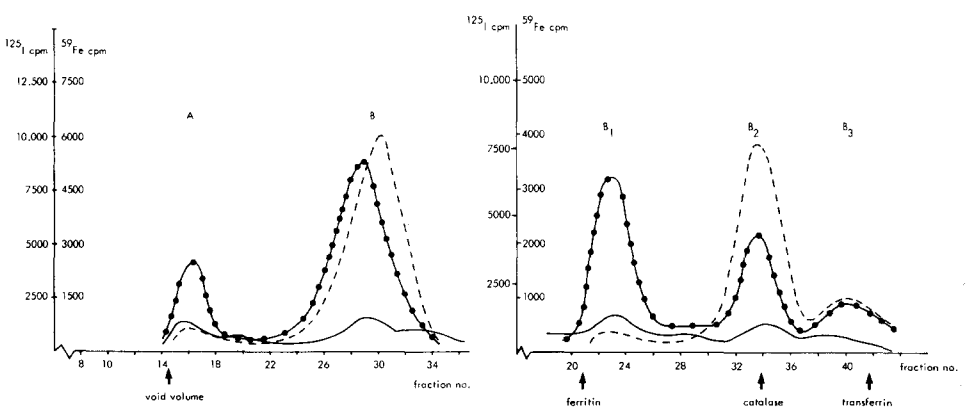

Fig. 1. Gel filtration of the solubilized reticulocyte membranes on Sepharose 2B. ●———●, ^{59}Fe; - - - - - -, ^{125}I; ———, protein.

Fig. 2. Rechromatography of the B component from the Sepharose 2B column on Sepharose 6B. ●———●, ^{59}Fe; - - - - - -, ^{125}I; ———, protein.

cells for specific binding of transferrin we incubated erythrocytes, unsealed reticulocyte ghosts and reticulocyte ghost fragments after solubilisation with Triton X-100, with doubly-labelled transferrin. The membranes or solutions were treated as described for the reticulocytes. The final chromatography on Sepharose 6B of the solutions resulted in an elution of most of the ^{59}Fe and ^{125}I activity at the B_3 position; only unsealed reticulocyte ghosts were able to bind a small amount of doubly-labelled transferrin specifically, as is shown in Fig. 3, as some of the ^{59}Fe and ^{125}I-activity is present at the B_2 position in unsealed ghosts. In all these experiments the B_1 fraction did not contain any radioactivity.

Sedimentation analysis

Triton X-100 was almost completely removed by means of Bio-beads. Sedimentation analysis of fraction B_2 showed heterogeneity, with, among others, the receptor complex with a sedimentation coefficient of approx. 7 S. Also a fraction with a sedimentation coefficient of 5.1 S was present, which probably represents unbound transferrin.

Anti-rat B_2-gammaglobulin and anti-total rat membrane gammaglobulin

The antibodies raised against the B_2 fraction react with among others, transferrin and the probable transferrin receptor. It appears that the anti-B_2 antibody reacts with more than two proteins, as is shown by four precipitation lines in an Ouchterlony diffusion plate. This means that the B_2 fraction is heterogeneous in its composition, which is in agreement with the results from the sedimentation analysis. The antibody fraction reacted neither with plasma nor intracellular components, nor with Triton X-100.

Incubation of the anti-B_2 immunoglobulin or the antibodies against other membrane components with a reticulocyte-rich cell suspension caused agglutination of this cell suspension.

As has been written in the Introduction, we wished to know whether blocking of the receptor with its antibody would be attended with an inhibition in transferrin binding and iron uptake.

It is obvious that the addition of anti-B_2 immunoglobulin or antibodies against the other membrane components except B_2 membrane fragments, would disturb the interpretation of the results obtained in two ways: firstly, by causing agglutination of the cell suspension; secondly, by binding and elminating transferrin from the incubation medium due to the presence of anti-transferrin in the immunoglobulin fractions.

In order to avoid these disturbances, the antibodies against B_2 and against the total reticulocyte membrane were purified by affinity chromatography over a Sepharose 4B-transferrin column, which removed the anti-transferrin part of the anti-B_2 antibodies. The purified anti-B_2 was split into F_{ab} and F_c fragments, as has been described elsewhere [32].

In order to study the blocking effect of F_{ab} fragments, a reticulocyte suspension was preincubated with several concentrations of F_{ab} fragments of anti-rat B_2 as has been described under Materials and Methods. After washing the cells extensively, the supernatant, after the last washing, did not contain free F_{ab} fragments. These preincubated cells had not been changed in shape. Immuno-

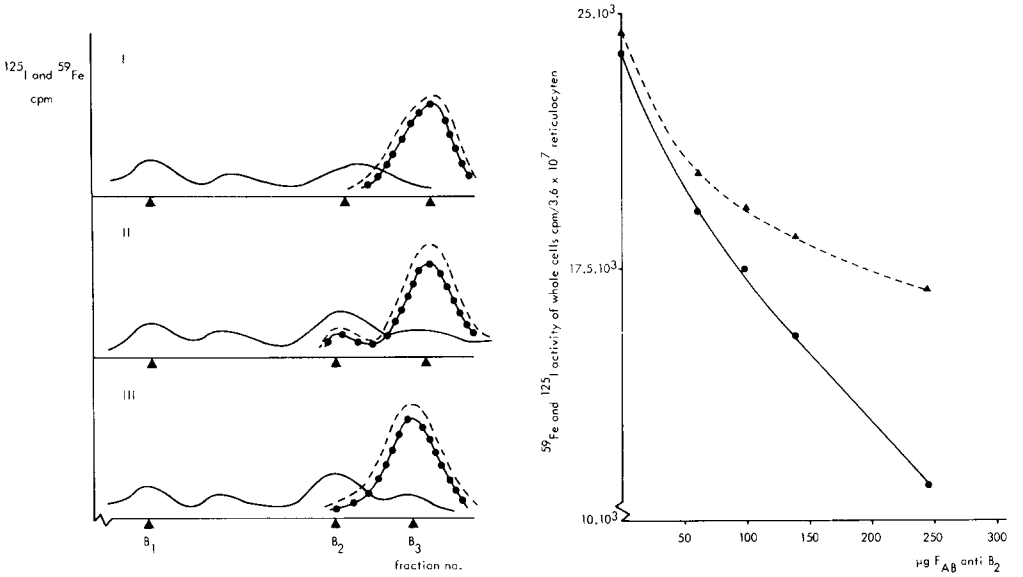

Fig. 3. Gel filtration of the solubilized membrane on Sepharose 6B. I, erythrocytes; II, ghosts; III, ghost fragments. The arrows indicate where B_1, B_2 and B_3 are normally eluted. ●———●, ^{59}Fe; - - - - - -, ^{125}I; ———, protein. Same indices on ordinate and abscissa as in Fig. 2.

Fig. 4. The binding of transferrin to the reticulocytes and the binding and uptake of iron into the reticulocytes after preincubation with several amounts of F_{ab} of anti-B_2. ▲- - - - -▲, [^{125}I]transferrin; ●———●, ^{59}Fe.

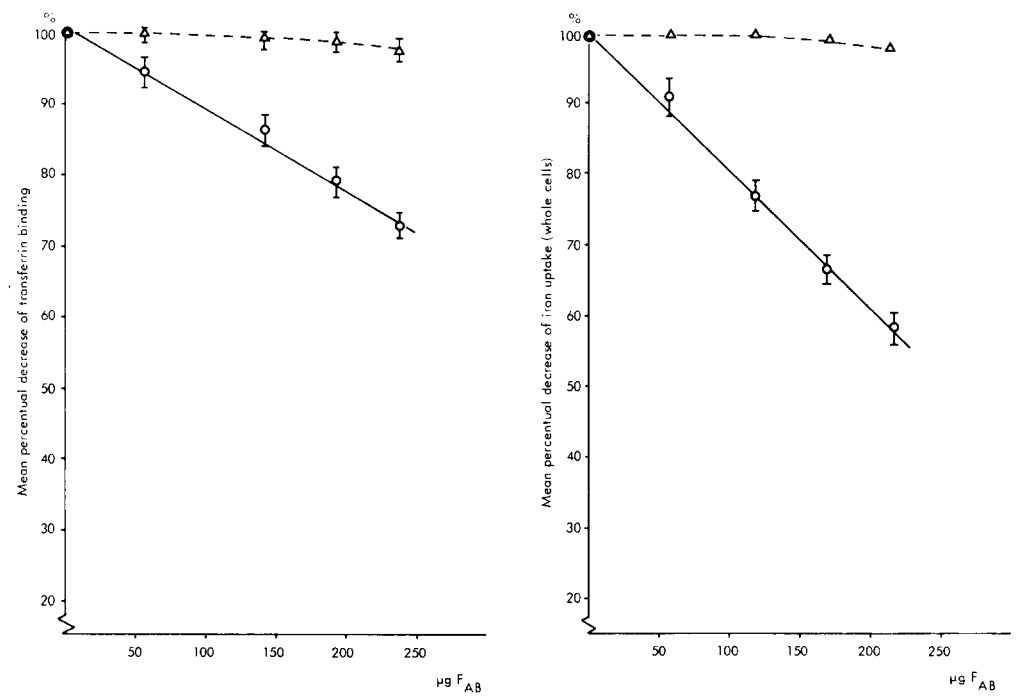

Fig. 5. The mean percentage decrease of transferrin binding to the reticulocyte after preincubation with several amounts of F_{ab} fragments of antibodies to rat IgG, to rat serum purified from transferrin and also to several membrane components except B_2 (△- - - - -△) and with F_{ab} fragments of antibodies to the B_2 component (○———○).

Fig. 6. The mean percentage decrease of iron uptake into the reticulocytes after preincubation with several amounts of F_{ab} fragments of antibodies to rat IgG, to rat serum purified from transferrin and also to several membrane components except B_2 (△- - - - -△) and after preincubation with F_{ab} fragments of antibodies to the B_2 component (○———○).

fluorescence studies showed that the F_{ab} fragments were bound to the cells.

After the pretreatment with F_{ab} fragments, the cells were incubated with double-labelled transferrin as has been described. One specimen of the same reticulocyte cell suspension was incubated directly with transferrin, without preincubation with the F_{ab} fragments of anti-B_2 immunoglobulin. These cells were treated in exactly the same way as the preincubated cells.

All incubation experiments were performed in duplicate. The transferrin binding and iron uptake was determined by measuring the ^{125}I and ^{59}Fe activities. The results are presented in Fig. 4. It is obvious that there is a decrease in transferrin binding and iron uptake, which is dependent on the concentration of the F_{ab} fragments of anti-B_2 in the preincubation medium.

The decrease in iron-uptake is more pronounced than the decrease in transferrin binding. In Figs. 5 and 6, the mean percentage decrease in transferrin binding and iron uptake for six experiments is presented. In order to investigate whether these decreases were not a nonspecific result due simply to the preincubation with a protein, 50% of a reticulocyte cell suspension was incubated with the F_{ab} fragments of IgG, with F_{ab} fragments of anti-rat serum from which anti-transferrin had been removed, or with F_{ab} fragments of antibodies against the other membrane components except B_2.

The other 50% of the reticulocytes were preincubated with the F_{ab} fragments of anti-B_2. Addition of the antibodies, first mentioned, had no effect on iron uptake and transferrin binding, although it was shown by immunofluorescence studies that the F_{ab} fragments of antibodies against other membrane components did bind to the membrane. It is clear that the inhibition of the

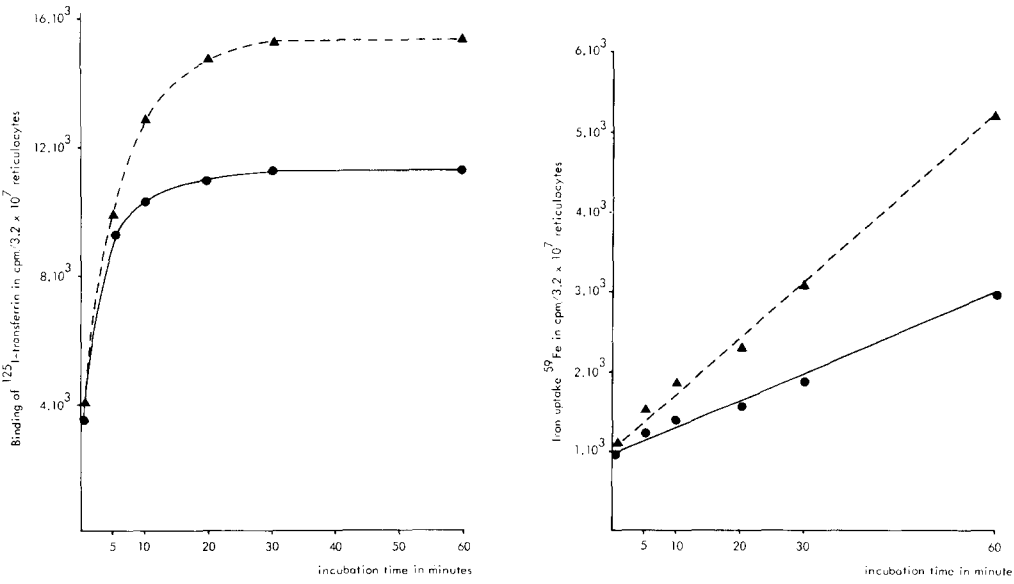

Fig. 7. The binding of transferrin to the reticulocyte during variable incubation times, ▲- - - - -▲, normal reticulocytes; ●———●, with F_{ab} fragments from B_2 preincubated reticulocytes.

Fig. 8. The uptake of iron into the reticulocyte during variable incubation times. ▲- - - - -▲, normal reticulocytes; ●———●, with F_{ab} fragments from B_2 preincubated reticulocytes.

transferrin binding and iron uptake by F_{ab} fragments of the anti-B_2 immunoglobulin is specific and significant (Figs. 5,6). There was a possibility that the type of process of transferrin binding and iron uptake had changed by the addition of F_{ab} fragments to preincubated cells, and that transferrin was bound and iron taken up by another process. For that reason, 50% of a reticulocyte cell suspension (suspension I) was incubated with 200 μg of F_{ab} fragments of anti-B_2. The other half of reticulocytes (suspension II) was treated similarly with respect to the preincubation time and temperature, but without F_{ab} fragments in the medium. Portions of the suspensions I and II were incubated with doubly-labelled transferrin during periods of 5, 10, 20, 30 and 60 min. After this incubation time, the reaction between the cells and transferrin was stopped (see Materials and Methods). The uptake of iron and the transferrin binding was determined by counting the radioactivity.

The results are shown in Figs. 7 and 8. It was shown that the type of process by which transferrin was bound to the cell and iron was taken up, had not changed by preincubation with the F_{ab} fragments. Only the amount of free receptor sites has been diminished.

Discussion

Although much information has been obtained about the process of iron uptake by immature erythroid cells, the exact mechanism is still not clearly understood. Kinetic studies with transferrin and reticulocytes showed that a receptor for transferrin on the cell membrane may play an important role in the initial step of the process. However, the real structure and functions of this receptor are unknown. This study showed that there exists a striking resemblance in membrane components involved in transferrin binding and iron uptake by rat reticulocytes and by human reticulocytes, which emphasizes the specificity of the membrane components described and of their functions.

The only difference between rat and human membranes in this respect is the nonspecific transferrin binding membrane component, B_3, and the fact that transferrin can dissociate from the solubilized receptor in rat reticulocytes. The function of the components A and B_1 in the process of iron uptake is still unknown. These components may function as intermediates between the transferrin receptor and intracellular proteins.

As in human reticulocytes, our B_2 component probably represents the transferrin · receptor complex, with a molecular weight of approx. 230 000 or lower. Although it seems reliable to presume that the B_2 component does contain the transferrin · receptor complex, sedimentation analysis and anti-B_2 antibody production showed that the composition of this B_2 component is heterogeneous and that the B_2 fraction may contain components such as Triton X-100 which do not form part of the receptor but are bound to it. Taking into account these observations it is likely that the molecular weight of the receptor for transferrin is lower than the present known molecular weights.

The fluorescence studies showed that, during the process of iron uptake into erythroid bone-marrow cells in vivo, transferrin is bound at the outside of the cell membrane. This observation makes it likely that the receptor for transferrin is localised at the outside of the cell membrane.

It was shown that for specific reaction between transferrin and the recep-

tor followed by loss of iron from transferrin it is necessary that transferrin reacts with intact immature red cells. Besides the intact immature red cells, only the unsealed reticulocyte ghosts could bind some transferrin specifically without loss of iron from the transferrin. Although results from the incubation experiments make it likely that component B_2 does contain the transferrin receptor complex, there is no proof, nor any indication, of its physiological role, in these experiments.

Purified F_{ab} fragments of antibody against B_2 caused specific, concentration dependent, inhibition of transferrin binding and iron uptake. This is evidence that the B_2 component really contains the receptor for transferrin, especially as antibodies against other membrane components did not cause any inhibition of transferrin binding and iron uptake. The experiments in which the influence of the time of incubation on the inhibition was investigated showed that the type of process by which transferrin is bound and iron is taken up by the cells had not been changed by preincubation with purified F_{ab} fragments of anti-B_2. The curves are of the same type. There still exists an initial adsorbance phase, a phase of specific binding and an equilibrium phase. The equilibrium phase was reached at 10—20 min (normally at 20—30 min), indicating that there are less free receptor places after preincubation with F_{ab} fragments. Also, we did not find any evidence for an increase of nonspecific binding of transferrin after reducing the number of free receptors. The more pronounced decrease in iron binding and iron uptake in comparison with the decrease in transferrin binding (10%) can be explained by assuming that the blocking of a receptor prevents the process of pinocytosis of neighbouring $^{59}Fe \cdot$ transferrin receptors, with the hypothesis that transferrin is taken up intracellularly [20,21]. Blocking the receptor may also inhibit the function of membrane components (B_1) which are concerned with iron transport from the neighbouring receptor through the membrane to intracellular components, with the hypothesis that transferrin is not taken up by the cell.

The results for rat and human reticulocytes agree very well with data concerning the membrane components A, B_1 and B_2, but not with data for rabbit reticulocyte membrane components as have been described by Leibman [25]. This difference may be caused by species differences [25,38].

References

1 Jandl, J.H., Inman, J.K., Simmons, R.L. and Allen, D.W. (1959) J. Clin. Invest. 38, 161—185
2 Jandl, J.H. and Katz, J.H. (1963) J. Clin. Invest. 42, 314—326
3 Morgan, E.H. and Laurell, C.B. (1963) Brit. J. Haematol. 9, 471—483
4 Baker, E. and Morgan, E.H. (1969) Biochemistry 8, 1133—1141
5 Baker, E. and Morgan, E.H. (1969) Biochemistry 8, 2954—2958
6 Kornfeld, S. (1968) Biochemistry 7, 945—954
7 Morgan, E.H. and Baker, E. (1969) Biochim. Biophys. Acta 184, 442—454
8 Edwards, S.A. and Fielding, J. (1971) Brit. J. Haematol. 20, 405—416
9 Morgan, E.H. (1971) Biochim. Biophys. Acta 244, 103—116
10 Baker, E. and Morgan, E.H. (1971) J. Cell. Physiol. 77, 377—384
11 Lane, R.S. (1972) Brit. J. Haematol. 22, 309—317
12 Eyged, A. (1973) Biochim. Biophys. Acta 304, 805—813
13 Hemmaplardh, D., and Morgan, E.H. (1974) Biochim. Biophys. Acta 373, 84—99
14 Hemmaplardh, D., Kailis, S.G. and Morgan, E.H. (1974) Brit. J. Haematol. 28, 53—65
15 Aisen, P. and Leibman, A. (1973) Biochim. Biophys. Acta 304, 797—804
16 Hemmaplardh, D. and Morgan, E.H. (1976) Biochim. Biophys. Acta 426, 385—398

17 Princitto, J.V., Rubin, M., Shashoty, G.C. and Zapolski, E.J. (1964) J. Clin. Invest. 43, 825—833
18 Speyer, B.E. and Fielding, J. (1974) Biochim. Biophys. Acta 332, 192—200
19 Fielding, J. and Speyer, B.E. (1974) Biochim. Biophys. Acta 336, 387—396
20 Morgan, E.H. and Appleton, T.C. (1969) Nature 223, 1371—1372
21 Appleton, T.C., Morgan, E.H. and Baker, F. (1971) in The Regulation of Erythropoiësis and Haemoglobin Synthesis (Travnicek, T. and Neuwirt, J., eds.), pp. 310—315, Universita Karlova, Praha
22 Martinez-Medellin, J. and Schulman, H.M. (1972) Biochim. Biophys. Acta 264, 272—284
23 Sly, D.A., Grohlich, D. and Bezkorovainy, A. (1975) Proteins of Iron Storage and Transport in Biochemistry and Medicine (Crighton, R.R., ed.), 141—145, Elsevier, Amsterdam
24 Van Bockxmeer, F., Hemmaplardh, D. and Morgan, E.H. (1975) Proteins of Iron Storage in Biochemistry and Medicine (Crichton, R.R., ed.), pp. 111—119, Elsevier, Amsterdam
25 Leibman, A. and Aisen, P. (1977) Biochemistry 16, 1268—1272
26 Van Bockxmeer, F.M. and Morgan, E.H. (1977) Biochim. Biophys. Acta 468, 437—450
27 Verhoef, N.J., Kremers, J.H.W. and Leijnse, B. (1973) Biochim. Biophys. Acta 304, 114—122
28 Verhoef, N.J. and Noordeloos, P.J. (1977) Cli. Sci. Mol. Med. 52, 87—96
29 Dodge, J.T., Mitchell, C. and Hanahan, D.J. (1963) Arch. Biochem. Biophys. 100, 119
30 Van Eijk, H.G. and Van Noort, W.L. (1976) J. Clin. Chem. Clin. Biochem. 14, 475—478
31 Van Eijk, H.G. (1966) Biochim. Biophys. Acta 127, 241—243
32 Franklin, E.C. (1969) J. Clin. Invest. 39, 1933
33 Katz, J.H. (1961) J. Clin. Invest. 40, 2143—2152
34 Verhoef, N.J. and Van Eijk, H.G. (1975) Cli. Sci. Mol. Med. 48, 335—340
35 Light, N.D. (1977) Biochim. Biophys. Acta 495, 46—57
36 Holloway, P.W. (1973) Anal. Biochem. 53, 304—308
37 Van der Heul, C., Kroos, M.J. and van Eijk, H.G. (1977) in Proteins of Iron Metabolism (Aisen, P., Brown, E.B., Fielding, J. and Crichton, R.R., eds.), pp. 299—304, Grune and Stratton, New York
38 Brown, E.B. (1977) Blood 50, 1151—1152