THE INTERACTION OF LECTINS WITH THE SURFACE OF DIFFERENTIATING ERYTHROLEUKAEMIC CELLS

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SUMMARY

Friend erythroleukaemic cells can be induced to mature along the erythroid differentiation pathway when an inducing agent such as dimethyl sulphoxide is included in the medium. In the absence of the inducing agent, the 707B line of Friend erythroleukaemic cells is highly agglutinable by the lectins concanavalin A or wheat germ agglutinin. However, 48 h after the induction of differentiation, there is a marked decrease in the agglutination of the cells in the presence of either lectin. This suggests that early in differentiation a change occurs in the cell membrane preceding the onset of globin synthesis which starts approximately 72 h after induction. The change in agglutination by concanavalin A also occurs in the presence of reagents which do not induce haemoglobin synthesis in the 707B line of Friend erythroleukaemic cells but which are able to stimulate the synthesis of this protein in other erythroleukaemic cell lines.

The reduction in the agglutinability of the differentiating cells does not seem to result from a reduction in the number of concanavalin A receptors on the cells, nor does it reflect a change in the clustered distribution of concanavalin A receptors in the differentiating cells. Both the control and dimethyl sulphoxide-induced cells show a similar patchy distribution of ferritin-labelled concanavalin A when examined by electron microscopy.

Polyacrylamide gel electrophoresis shows little change in the total pattern of protein synthesis by control and differentiating cells when pulse-labelled with [35S] methionine. However, use of 125I-labelled concanavalin A to stain polyacrylamide gels, on which the total proteins of control and differentiating cells had been separated, revealed a profound change in the composition of the concanavalin A-binding proteins. The control, undifferentiated cells contained eleven or more classes of concanavalin A-binding glycoproteins, many of which stained to a lesser degree as the cell density increased. After the onset of differentiation, 2 new concanavalin A-binding glycoproteins appeared within 48 h. One of these proteins has a molecular weight in excess of 180,000 while the other migrated with an apparent molecular weight of approximately 100,000. After erythroid differentiation had progressed for 120 h, these newly synthesized glycoproteins became the major concanavalin A-binding proteins of the erythroleukaemic cells.

INTRODUCTION

Friend erythroleukaemic (FL) cells are thought to have arisen by the transformation of a murine, committed erythroid precursor cell. In culture these cells proliferate rapidly and do not progress very far along the erythroid differentiation pathway. (For review see Harrison, 1977.) However, when one of a variety of inducing agents (for example, dimethyl sulphoxide (DMSO)) is added to the medium, the actively proliferating cells can be switched to differentiate and acquire some of the characteristics of the mature erythrocyte. On induction of differentiation, growth and proliferation are reduced and after approximately 72 h the cells start to synthesize
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haemoglobin, eventually turning red (Friend, Scher, Holland & Sato, 1971). Prior to
the onset of globin synthesis, several other changes typical of erythroid differentiation
occur and some of these involve the plasma membrane. For example, glycophorin and
spectrin are synthesized (Eisen, Bach & Emery, 1977a). These 2 proteins are major
components of the erythrocyte membrane, the former being a transmembrane gly-
coprotein (Bretscher, 1971) and the latter a constituent of the peripheral cytoskeleton
which lines the cytoplasmic surface of the membrane (Scheetz & Singer, 1977). In
contrast to the increase in these proteins, the amount of H2 antigen on the cell surface
decreases (Arndt-Jovin et al. 1976) while other changes result in an alteration of
membrane viscosity and permeability (Arndt-Jovin et al. 1976) and the interaction
of the cells with plant lectins which bind to surface oligosaccharides (Eisen et al. 1977b).
Eisen and his colleagues showed that the F4N line of FL cells is not agglutinable by
a variety of lectins when actively proliferating. Almost immediately after the induction
of differentiation, however, the agglutinability increases. Later in differentiation, the
agglutination falls once again.

We have investigated the changes that occur in the surface of differentiating FL
cells (line 707B) as detected by the lectins concanavalin A (Con A) and wheat germ
agglutinin (WGA) in order to determine whether alterations in lectin-mediated
agglutination reflect the synthesis and insertion into the membrane of new glyco-
proteins. In this report we show that the 707B line of FL cells is highly agglutinable
by either lectin. After 48 h growth in the presence of 230 mM DMSO, the agglutin-
ability by either Con A or WGA is reduced. The reduction in the Con A-mediated agglutin-
ability is not the result of a decrease in the number of Con A receptors or a change in
the ability of the receptors to form small clusters in the presence of the lectin. Correlat-
ing with the change in agglutinability, however, is a substantial alteration in the com-
plement of the individual classes of Con A receptors in the cell.

MATERIALS AND METHODS

Biochemicals and chemicals

Concanavalin A (type IV), wheat germ agglutinin, ferritin and ouabain were purchased from
the Sigma Chemical Co. London. Ferritin-conjugated Con A (for-Con A) was either prepared
according to the method of de Petris (1976) or purchased from Calbiochem, San Diego, Calif.
Osmium tetroxide, butyric acid and DMSO were bought from BDH Ltd., Poole, England.
Other materials for electron microscopy came from Taab Laboratories, Reading, England.
[^35S]methionine (1380 Ci/mmol) and Na[^125]I (11–17 mCi/μg I) were purchased from the
Radiochemical Centre, Amersham.

Cell lines and growth conditions

707B, 707T and FW lines of FL cells were obtained from Dr T. Rutherford, Radcliffe Infirmary,
Oxford, England. (For further details of the derivation of these cell lines see Harrison
et al. 1978.)

All cells were grown in suspension culture in 75-cm² Nunc tissue culture flasks using Ham’s
F-10 medium (Flow Labs. Irvine, Scotland) supplemented with 5% foetal calf serum (Gibco-
Biocult, Glasgow, Scotland) and BME amino acids (Gibco-Biocult).

For induction of erythroid differentiation, cells were subcultured to a density of 10⁶ cells per
ml. After growth for 24 h, DMSO was added to the medium to give a final concentration of
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230 mM. Alternatively medium, containing other potential inducers in a concentrated form, was resterilized by membrane filtration and added to the cells 24 h after subculture.

Agglutination by Con A and WGA

10⁶ control or differentiating cells were washed in Dulbecco's phosphate-buffered saline lacking CaCl₂, pH 7.4 (PBS) and resuspended in PBS containing the lectin at a final concentration of 10-100 μg per ml. The final incubation volume was 200 μl. After 30 min at 37 °C, the cells were harvested by centrifugation at 670 g in a bench centrifuge and resuspended in 100 μl PBS. One drop of the suspension was applied to a haemocytometer slide and the numbers of clumped and single cells were counted. At least 200 cells were counted in duplicate in each agglutination assay. The number of agglutinated cells was determined as a percentage of the total number of cells and expressed on the 'plus' system of de Petris (1976): 0-12.5 % agglutinated cells were scored as zero agglutination, 12.6-56 % as 1+, 56.1-79 % as 2+, 79-1-89.9 % as 3+ and 90-100 % as 4+. The agglutination assays were performed in duplicate.

Control incubations were carried out in the presence of 0.1 M α-methyl mannoside (for Con A) and 0.1 M N-acetyl glucosamine (for WGA).

Lectin receptor number determination

Con A was labelled with Na¹²⁵I by the lactoperoxidase method described by Arndt-Jovin & Berg (1971). Duplicate aliquots of 10⁶ control or differentiating cells were suspended in 1 ml PBS containing 0-500 μg¹²⁵I-Con A. After 15 min at 4 °C, the incubation mixture was layered onto a solution of 10 % bovine serum albumin (BSA) in PBS and the cells collected by centrifugation at 670 g on a bench centrifuge at 4 °C. The cell pellet was washed in cold PBS and precipitated with cold 10 % trichloroacetic acid and collected on Millipore filters. The bound radioactivity was determined by scintillation counting.

A further set of duplicate incubations were carried out as above except that 0.1 M α-methyl mannoside was included. These incubations served as controls for the non-specific adherence of Na¹²⁵I-Con A to the cells.

Ferritin-Con A labelling of Cells

5 x 10⁶ control or differentiating FL cells were harvested and washed 4 times in PBS containing 1 % BSA by centrifuging at 670 g for 5 min at room temperature. The cells were then resuspended on 0.4 ml PBS/1 % BSA containing Con A, as the fer-Con A conjugate, at a final concentration of 125 μg/ml. The cell/fer-Con A mixture was incubated for 30 min at 37 °C and then diluted with 2 ml PBS/1 % BSA and centrifuged for 10 min at 1500 g on a bench centrifuge. The supernatant was carefully removed and the pellet of cells was resuspended in 3 % glutaraldehyde/1 % formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). The cells were then incubated with 1 % osmium tetroxide and processed for electron microscopy using uranyl acetate and lead citrate staining.

In control experiments, cells were fixed with 2 % glutaraldehyde for 2 h prior to incubation with fer-Con A or were incubated with fer-Con A in the presence of 0.1 M α-methyl mannoside.

Thin sections were viewed in a Siemens electron microscope and the micrographs printed on Ilfospeed 5 high-contrast paper.

Metabolic labelling

10⁶ cells were harvested and resuspended in 25 ml Ham’s F-10 medium lacking methionine and supplemented with 25 μCi [³⁵S] methionine. The cells were incubated for 2 h at 37 °C, harvested, washed twice in PBS and dissolved for polyacrylamide gel electrophoresis as described below.
Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Unlabelled or [35S] methionine-labelled cells were dissolved in a solution of 5% sodium dodecyl sulphate (SDS) and 1% 2-mercaptoethanol and boiled for 2 min. The dissolved proteins were separated on slabs of 8.5% polyacrylamide using the buffer system of Laemmli (1970). After electrophoresis, the gels were fixed and stained with coomassie blue. The gels containing radioactively labelled proteins were dried and autoradiographed.

Gels on which unlabelled total cell proteins had been separated, were washed free of fixative by agitating for 24 h in several changes of PBS. The gel was then immersed in a solution of 125I-Con A prepared as described above. After 3 days during which the gel was occasionally agitated, the lectin solution was removed and the gel was washed for 48 h in a continuous stream of PBS. The washed gel was dried and autoradiographed (Burridge, 1976).

Haemoglobin determination

Approximately 10^7 cells were washed in cold PBS and lysed in 1 ml of a solution containing 0.15 M NaCl, 3.5 mM Mg acetate, 0.5% nonidet P-40 and 10 mM Tris-HCl (pH 7.4). After 15 min, the nuclei were removed by centrifugation for 10 min at 1500 g and the optical density of the post-nuclear supernatant was measured at 425, 414 and 403 nm. The absorption maximum of haemoglobin at 414 nm was corrected for light scattering using the formula: 

\[ A_{414}^{\text{corrected}} = 3 \times (A_{414} - 0.5 \times A_{403} - 0.5 \times A_{425}) \]

A corrected optical density at 414 nm of 1.0 is equivalent to 0.13 mg haemoglobin per ml (Rutherford & Weatherall, 1979).

RESULTS

Lectin agglutination of FL cells during erythroid differentiation

FL cells were grown in Ham’s F-10 medium in suspension culture for a period of 6 days. On each day cells were harvested and tested for their agglutinability in the presence of either Con A or WGA. At all of the concentrations of the lectins tested (which ranged from 100 to 5 μg per ml in a 200-μl incubation containing 10^6 cells), the control cells formed large aggregates of 20 or more cells (Fig. 1). In some experiments in which a low concentration of Con A (10 μg per ml) was used (Fig. 1 – top right), the agglutinability of the control cells was observed to be less than 100% at zero time and after 120 h of growth. At these times the cells were in lag or approaching stationary phase respectively, and this observation is in accord with several findings that actively proliferating cells are more agglutinable than resting cells. (For a review see Brown & Hunt, 1978.)

Immediately after the addition of DMSO (final concentration 230 mM) to the growth medium, the cells are found to be as agglutinable by either lectin as are the control cells (Fig. 1). After a further 24 h of growth in the presence of DMSO they are still highly agglutinable. During the period between 24 and 48 h after the addition of DMSO, however, their agglutinability falls in the presence of either Con A or WGA. The clumps of cells formed after growth for 48 h in DMSO-containing medium were much smaller than those formed with the lectins by control cells and 40–50% of the cells were not agglutinated at all. In all experiments, the agglutination was shown to be inhibited by the appropriate competitive sugar, α-methyl mannoside in the case of Con A and N-acetyl glucosamine in the case of WGA.

In order to test whether the reduction in agglutinability observed 48 h after the induction of differentiation was a result of DMSO alone and not related to its dif-
ferentiation-induction properties, the effect of other potential inducers on Con A-mediated agglutinability was tested. The additional inducers used have both been reported to cause erythroid differentiation in one or more FL cell lines. 707B FL cells were grown in the presence of ouabain (Bernstein, Hunt, Critchley & Mak, 1976b) or butyric acid (Leder & Leder, 1975) and their agglutinability by Con A was tested after

Fig. 1. Agglutination of control and differentiating FL cells by: 100 µg Con A per ml (top left); 10 µg Con A per ml (top right); 100 µg WGA per ml. (bottom left); and 5 µg WGA per ml (bottom right). 10^6 control (●) or differentiating (○) cells were harvested on each day after addition of DMSO to initiate the differentiating cultures and their agglutination was assayed by incubation for 30 min at 37 °C with the appropriate concentration of the lectin.

48 h. The remainder of the culture that was not used in the agglutination tests was grown for a further 48 h in the presence of the potential inducer and the content of haemoglobin was determined. Both ouabain and butyric acid reduced the proliferation of FL cells in a manner similar to that of DMSO but only DMSO, of the potential inducers used, caused the induction of haemoglobin synthesis within 96 h (Table 1). However, all three agents produced a marked fall in the agglutinability of the cells. This suggests that the early decrease in the agglutination is not a peculiarity of DMSO
alone. The observation that only DMSO leads to the synthesis of detectable amounts of haemoglobin indicates that the surface change detected by the lectins early in the process of differentiation is not an event that automatically leads to a later synthesis of haemoglobin.

Whilst a decrease in lectin-mediated agglutinability does not necessarily lead to haemoglobin synthesis, it is possible that such a change must always occur for haemoglobin synthesis to take place. Two other FL cell lines were tested for their agglutinability by various concentrations of Con A 48 h after the induction of erythroid dif-

Table 1. Effect of potential inducers of erythroid differentiation on the Con A-mediated agglutination of FL cells

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Concentration, mM</th>
<th>Cells/ml (x 10^-4) at 96 h after induction</th>
<th>Agglutination 48 h after induction</th>
<th>Haemoglobin (µg/10^7 cells) 96 h after induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>450</td>
<td>4+</td>
<td>—</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.1</td>
<td>365</td>
<td>2+</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>395</td>
<td>3+</td>
<td>—</td>
</tr>
<tr>
<td>Butyrate</td>
<td>1.0</td>
<td>56</td>
<td>2+</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>260</td>
<td>1+</td>
<td>—</td>
</tr>
<tr>
<td>Ouabain</td>
<td>0.1</td>
<td>36</td>
<td>3+</td>
<td>—</td>
</tr>
</tbody>
</table>

After 48 h growth in the presence of the potential inducing agent, FL cells were harvested and tested for their agglutinability by Con A at a concentration of 100 µg/ml. The remainder of each cell culture was grown for a further 48 h, harvested and the cell number and haemoglobin content determined.

Table 2. Con A-mediated agglutination of FL cell variants

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Addition</th>
<th>Agglutination 48 h after addition</th>
<th>Haemoglobin (µg/10^7 cells) 96 h after addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>707T</td>
<td>None</td>
<td>4+</td>
<td>—</td>
</tr>
<tr>
<td>707T</td>
<td>230 mM DMSO</td>
<td>4+</td>
<td>30</td>
</tr>
<tr>
<td>FW</td>
<td>None</td>
<td>4+</td>
<td>—</td>
</tr>
<tr>
<td>FW</td>
<td>1.75 mM butyric acid</td>
<td>4+</td>
<td>—</td>
</tr>
<tr>
<td>FW</td>
<td>1.75 mM butyric acid + 0.1 mM haemin</td>
<td>4+</td>
<td>47</td>
</tr>
</tbody>
</table>

Each cell line was grown in the presence or absence of the appropriate agent. After 48 h they were harvested and tested for their agglutinability by Con A at a concentration of 100 µg/ml. After a further 48 h the remainder of the cultures was harvested and the haemoglobin content determined.
ferentiation. The 707T line is a thioguanidine-resistant derivative of the parental cells of the 707B line and also inducible by DMSO. Like the 707B line these cells are highly agglutinable when proliferating in the absence of inducing agent. However, no change takes place in the agglutinability of these cells during the course of differentiation at any of the concentrations of Con A used (Table 2). The FW cell line is not induced to differentiate by DMSO but is induced by a combination of butyric acid and haemin. Once again, no decrease in agglutinability of these cells occurs on induction. These results suggest that in at least the 707T and FW lines, the membrane change detected by a fall in lectin-mediated agglutinability is not a prerequisite of the later induction of haemoglobin synthesis.

The number of Con A receptors on the FL cell surface during erythroid differentiation of the 707B line

Incubations containing approximately $10^6$ cells and 0–500 μg $^{125}$I-labelled Con A were carried out for 15 min at 4 °C. The cells were then separated from the unbound lectin by centrifugation through a cushion of 10% BSA and washed in PBS. The

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Growth period, h</th>
<th>Receptors per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>48</td>
<td>$2.2 \times 10^7$</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>$2.9 \times 10^7$</td>
</tr>
<tr>
<td>230 mM DMSO</td>
<td>48</td>
<td>$1.9 \times 10^7$</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>$3.5 \times 10^7$</td>
</tr>
</tbody>
</table>

Cells were subcultured to a density of $10^6$ cells per ml and grown either in the presence or absence of inducer for 48 or 120 h. $10^6$ cells were then incubated with several concentrations of $^{125}$I-Con A and the maximum number of molecules of Con A that bound to the cell surface determined.

The distribution of Con A receptors over the surface of 707B FL cells

It has been widely reported that lectins cause a redistribution of their receptors in the membrane of highly agglutinable neoplastic cells but do not have this effect on the receptors of the non-agglutinable normal counterparts. (For a review see Brown & Hunt, 1978.) It is usually found that the receptors of neoplastic cells are patched in the presence of the lectin while those of normal cells are randomly distributed. To determine whether such a difference in receptor redistribution by the lectin (which
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itself reflects a difference in receptor mobility) could explain the reduced agglutinability of differentiating FL cells, fer-Con A was used to label control and DMSO-induced cells. Cells were subcultured and half were supplemented with DMSO. Daily after subculture, control and induced cells were harvested, washed with PBS and then incubated with fer-Con A for a period of 30 min. After separation of the cells from unbound fer-Con A, they were processed for thin-section electron microscopy.

Fig. 7. Distribution of ferritin-labelled Con A on the surface of control FL cells fixed for 2 h in 2% glutaraldehyde prior to incubation with the lectin. × 40000.

The distribution of fer-Con A on the surfaces of highly agglutinable control cells 24 h after subculture is shown in Fig. 2. The fer-Con A is distributed in patches interspersed with ferritin-free areas. The electron-dense lectin conjugate appears to be particularly concentrated between the agglutinated cells and is also especially dense in the area of membrane through which a virus is budding (Fig. 2). After 24 h growth in the presence of 230 mM DMSO, FL cells show a similar patchy distribution of lectin. Forty-eight hours after subculture, the distribution of fer-Con A on the control cells remains patchy (Fig. 3). Cells grown for a similar period with DMSO are no longer highly agglutinable and fall into 2 types: those that form small clumps of 2 or 3 cells and those that do not adhere at all. The cells that form small clumps show a similar distribution of receptors to that of the control cells (Fig. 4) as do the non-agglutinable cells (Fig. 5).

The binding of fer-Con A to the cell surface is inhibited by the presence of 0.1 M
The patchy distribution of lectin receptors observed on the cell surface may be the result of the formation of receptor lattices by the multivalent lectin. This can occur if the receptors are free to move in the plane of the membrane. In order to determine whether this is in fact the case, cells were fixed with glutaraldehyde before incubation with fer-Con A. Fig. 7 shows that the fixed cells still have patches of lectin receptors on their surfaces.

The Con A receptors of control and differentiating 707B FL cells

The changes that take place in the synthesis of FL cell proteins after induction of differentiation were investigated by pulse labelling control and DMSO-induced cells daily after the addition of inducing agent to the latter cell cultures. The \[^{35}S\] methionine-labelled proteins made on each day were analysed by SDS-polyacrylamide gel electrophoresis. No difference could be seen in the proteins made by the control and induced cells during the course of the experiment when the proteins were separated on 8.5% polyacrylamide gels (Fig. 8A). Gels containing a higher percentage of acrylamide showed that among the proteins of lower molecular weight not resolved by the 8.5% gels, the only change in the DMSO-induced cells when compared to control cells was the onset of globin synthesis 72 h after addition of DMSO. (Two-dimensional polyacrylamide gel electrophoresis in which the proteins were first separated according to their isoelectric points and then, in the second dimension, according to their molecular weights (O’Farrell, 1975) also showed only a few differences in the proteins synthesized by control and differentiating cells; R. Hunt and L. Marshall – manuscript in preparation.) The above methods, however, only reveal the major proteins synthesized by the cells. It is likely that the plasma membrane proteins constitute only a few per cent of the total cell proteins, and changes are therefore unlikely to show up on SDS–polyacrylamide gel electrophoresis of \[^{35}S\] methionine-labelled proteins. Furthermore, many membrane proteins are glycosylated and may form diffuse bands on gel electrophoresis.

In order to discriminate lectin receptors from the many other proteins that are resolved by SDS–polyacrylamide gel electrophoresis, FL cells were harvested after growth for 48 and 120 h in the presence or absence of 230 mM DMSO. The proteins of the unlabelled cells were then analysed on 8.5% polyacrylamide slab gels after which they were fixed and stained with coomassie blue. After washing away the fixative with PBS, the gels were immersed in a solution of \(^{125}\text{I}\)-labelled Con A for 2 days and then washed for 2 days in PBS to remove unbound lectin (Burridge, 1976). The \(^{125}\text{I}\)-Con A stained gels were dried and autoradiographed. These experiments showed that control FL cells have eleven or more classes of Con A-binding glycoproteins ranging in molecular weight from less than 25 000 to greater than 180 000. The 48-h and 120-h control cells were qualitatively similar although the intensity of the \(^{125}\text{I}\)-Con A staining of some of the bands was reduced in the cells that had grown to a higher density (Fig. 8B). DMSO-induction of erythroid differentiation leads to major changes in Con A-binding glycoproteins. By 48 h after the onset of differentiation, the relative
intensity of the staining of several bands is diminished while the intensity of the staining of a diffuse band with an apparent molecular weight of 100,000 is greatly increased (Fig. 8B, track 2). In addition, a band at the top of the gel representing high-molecular-weight material also appears. Cells which have been grown for 120 h in the presence of DMSO show a simplified spectrum of Con A-binding proteins compared to the corresponding control cells. (Compare Fig. 8B, track 4 with Fig. 8B, track 3.)

![Fig. 8. A. Synthesis of [35S] methionine-labelled proteins by control and differentiating FL cells. On each day after subculture, 10⁶ control and differentiating cells were pulse labelled and analysed by SDS-polyacrylamide gel electrophoresis using 8.5% slab gels. Track 1: control cells after 24 h growth in new medium. Track 2: differentiating cells after 24 h growth in the presence of 230 mM DMSO. Track 3: control cells after 48 h growth in new medium. Track 4: differentiating cells after 48 h growth in the presence of 230 mM DMSO. Track 5: control cells after 72 h growth in new medium. Track 6: differentiating cells after 72 h growth in the presence of 230 mM DMSO. B. Staining of FL cell proteins with 125I-labelled Con A. FL cells that had been grown for either 48 or 120 h in the presence or absence of 230 mM DMSO were analysed by electrophoresis on 8.5% polyacrylamide-SDS slab gels. Equal amounts of total cellular protein were applied to each track of the gel. After electrophoresis, the gels were fixed and incubated with radio-iodinated Con A. Track 1: control cells harvested 48 h growth. Track 2: differentiating cells after 48 h growth in 230 mM DMSO. Track 3: control cells harvested after 120 h growth. Track 4: differentiating cells harvested after 120 h growth in the presence of 230 mM DMSO. The vertical column of figures indicates apparent molecular weight of the labelled proteins (x 10⁶).]
The band at the top of the gel, the 100,000 Dalton protein and a protein migrating with an apparent molecular weight of 90,000 are now the major Con A-binding glycoproteins.

**DISCUSSION**

Actively proliferating FL cells of the 707B line are readily agglutinated by Con A or WGA. As little as 2 μg of either lectin in an assay containing 10⁶ cells is enough to cause complete aggregation of the cells into clumps of 20 or more cells. In this manner, 707B FL cells resemble other actively proliferating cells such as transformed fibroblasts (for review see Brown & Hunt, 1978) and cells of the male germ line (Letts et al. 1978).

FL cells can be switched from active proliferation to a process of maturation along the erythroid differentiation pathway by addition of one of several inducing agents to the growth medium. When erythroid differentiation occurs, DNA synthesis and cell division are reduced and finally cease altogether. Total protein synthesis also decreases but the synthesis of several new proteins characteristic of the mature erythrocyte is initiated. Among these are globin (Friend et al. 1971), spectrin (Eisen et al. 1977a) and glycophorin (Eisen et al. 1977a). Other changes that occur during differentiation include an alteration in the morphology of the cells (for a review see Harrison, 1977) and several changes in the surface membrane. Besides the synthesis of the 2 membrane proteins, spectrin and glycophorin, the microviscosity and permeability of the membrane change (Arndt-Jovin et al. 1976). Whether the change in the latter 2 properties is a result of the insertion of new proteins into the membrane is not known.

We have shown that an alteration in the lectin-mediated agglutination of the 707B line of FL cells takes place within 48 h of the initiation of erythroid differentiation and precedes the onset of globin synthesis. The observed reduction of agglutinability is from the formation of large cell clumps to the formation of small clumps with about half of the cells remaining unagglutinated. This is in marked contrast to the results of Eisen and his colleagues (1977b) who found that control FL cells of the F4N line were not agglutinated by Con A or WGA but became agglutinable almost immediately after addition of DMSO to the medium. It is probable that the differences between our results are due to the different lines of FL cells used.

The underlying mechanism of the change in agglutinability of differentiating FL cells is not known but is of great interest since it may reflect a fundamental change in the plasma membrane during the process of differentiation. In neither the 707B nor F4N line of cells, however, does this change reflect an alteration in the total number of Con A receptors.

A widely favoured explanation for changes in agglutination observed on transformation of fibroblasts is a change in the mobility of lectin receptors such that those of the agglutinable cells can form clusters in the presence of the multivalent lectin, thereby forming more stable intercellular cross-bridges. In contrast the non-agglutinable fibroblasts are supposed not to be able to form such stable interactions because of the inability of their receptors to form clusters. This type of explanation has received support from several studies but many exceptions have also been documented. (For a
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review see Brown & Hunt, 1978.) In FL cells there is no change in the distribution of
Con A receptors correlating with the change in agglutination during 6 days growth in
the presence of DMSO. In the control and agglutinable DMSO-induced cells, fer-
con A is distributed in patches and concentrated in that region of the cell surface that
is apposed to another cell. It is, however, possible that a major change on differentia-
tion, resulting in reduced agglutinability, is the loss of the ability to aggregate small
receptor patches into much larger aggregates which may be necessary for stable inter-
cellular adhesion. Whether this is the case is not shown by our studies. Experiments
with local anaesthetics suggest that there might indeed be an alteration in receptor
mobility during the differentiation of FL cells. These compounds inhibit erythroid
differentiation in the presence of DMSO (Bernstein, Boyd, Critchley & Lamb, 1976a)
and while their mode of action is unknown, they may increase membrane fluidity or
breakdown components of the intracellular cytoskeleton that ordinarily anchor inte-
gral glycoproteins (Poste, Papohadjopoulos, Jacobson & Vail, 1975). In either case,
the local anaesthetic should enhance the mobility of the lectin receptors thereby
allowing the membrane of DMSO-induced cells to mimic that of control uninduced
cells.

The major change that we have observed in the Con A receptors of FL 707B cells as
they differentiate is in their composition as revealed by SDS-polyacrylamide gel
electrophoresis. After growth to high cell density in DMSO-containing medium, 2
new proteins are the major Con A receptors in the cell. It is possible that the fall in
agglutinability reflects this change in receptor composition, for the presence of the new
glycoproteins by 48 h after the onset of differentiation correlates with the fall in
agglutinability. However, whether this is so remains to be discovered. There is
evidence (Eisen et al. 1977a) that spectrin synthesis is also initiated about 48 h after
the onset of differentiation. This protein is a component of the cytoskeleton which
has been suggested to restrict the mobility of integral glycoproteins in the plane of the
membrane (Elgsaeter, Shotton & Branton, 1976; Nicolson & Painter, 1973). It is
possible that spectrin and the new glycoproteins synthesized by differentiating cells
together form a network that impedes the free diffusion of proteins in the plane of the
bilayer, thereby reducing the ability of the lectin to agglutinate the differentiating
cells.

We have tested 2 other lines of FL cells for their agglutinability by Con A during
differentiation in order to determine whether the surface change that occurs in the
707B line of cells is common to other cells committed to erythroid differentiation.
Our results show that no such change occurs in the 2 cell lines tested, implying that the
alteration in the membrane that results in a reduction of Con A-mediated agglutin-
ability is not a necessary prerequisite in an ordered series of steps that lead to the final
onset of globin synthesis. Our results with inducers of differentiation of other FL
cell lines which do not promote differentiation in the 707B line show, in addition, that
the occurrence of the lectin-detected surface change does not necessarily imply that
the cells will differentiate as far as globin synthesis.

Lectin-mediated agglutination is, however, a very non-specific criterion for investi-
gating cell differentiation. It is possible that most FL cells undergo alterations in their
glycosylated membrane proteins (both with respect to identity and distribution/anchorage characteristics) but that only in the FL 707B line, of the cells that we have tested, does this fortuitously correlate with the gross change in lectin agglutinability. It is important to use more powerful techniques such as polyacrylamide gel electrophoresis and radio-lectin staining to elucidate more subtle changes.

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REFERENCES


The surface of erythroleukaemic cells


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