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# CHANGES IN SURFACE ARCHITECTURE DURING MURINE ERYTHROLEUKEMIA CELL DIFFERENTIATION AS DETECTED BY LECTIN BINDING AND AGGLUTINATION

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**Cell surface alterations occurred during murine erythroleukemia cell (clone 745) differentiation that were detected by both agglutination and lectin binding. Agglutination of erythroleukemia cells was produced by wheat germ agglutinin; whereas, concanavalin A, Ricin, soybean agglutinin and fucose-binding protein were either ineffective or much less efficacious. Treatment of leukemia cells with the inducer of erythroid differentiation dimethylsulfoxide (DMSO) caused a progressive accumulation of hemoglobin-containing cells in culture and a decrease in the rate of agglutination by wheat germ agglutinin, which began at 24 h after exposure to the polar solvent, reached a nadir at 48 h, and remained essentially constant thereafter. The binding of radioactive wheat germ agglutinin by untreated control erythroleukemia cells increased with time in culture, reaching a maximum value at 48 h, and decreased progressively thereafter. Although an increase in $^{3}$H-labeled wheat germ agglutinin binding also occurred in DMSO-treated cells, the level bound was significantly lower than that observed in control cells at 24–96 h. The treatment of erythroleukemia cells with various concentrations of DMSO resulted in a decrease in the number of wheat germ agglutinin receptor sites. Other inducers of differentiation (i.e., dimethylformamide, bis(acetyl)diaminopentane) also inhibited the rate of wheat germ agglutinin-induced agglutination of erythroleukemia cells while, in contrast, the inducer tetramethylurea did not. These studies indicate that membrane changes occur during differentiation and suggest that there may be more than one mechanism involved in the initiation of maturation which ultimately leads to the common pathway of erythroid development.**

## Introduction

The mechanism(s) by which various genes are expressed during a normal program of development or are suppressed in neoplastic cells is not understood. The Friend erythroleukemia appears to be a valuable model system to study the phenomena involved in the regulation of cell differentiation in that it represents a relatively pure population of virus-transformed mouse hematopoietic cells [1] that can be induced to maturate along the erythroid pathway by dimethylsulfoxide (DMSO) [2], and a large variety of other chemical agents [1]. The process of erythroid maturation of Friend cells is expressed by a number of morphological and biochemical changes which resemble normal erythropoiesis [1–5], with fully differentiated cells resembling ortho- or polychromatic normoblasts [2,3]. Under certain experimental conditions in culture, the normoblasts are able to be converted to enucleated red cells [6]. The mechanism involved in 'triggering' the differentiation

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process is unknown, although several possibilities have been considered [1].

The experiments presented in this paper were designed to characterize alterations in cell surface architecture that occur during interaction between chemical inducers of differentiation and erythroleukemia cells. Plant lectins were used as probes of cell surface structure; these agents, which recognize and bind to specific carbohydrate residues on the cell surface, have been employed to monitor the differentiation process in a number of clones of the Friend leukemia with somewhat different findings [7–9]. Thus, Fujinami et al. [7] and Eisen et al. [8] have reported an increase in the agglutinability of murine leukemia cells by phytohemagglutinin and concanavalin A, respectively, during erythroid maturation; whereas, more recently, Hunt and Marshall [9] using concanavalin A, and our laboratory in preliminary studies with wheat germ agglutinin presented in abstract form [10] have demonstrated a decrease in agglutination by plant lectins during the differentiation process. This paper formally reports our findings which demonstrate that wheat germ agglutinin-induced agglutination of untreated control erythroleukemia cells was reduced by treatment with either DMSO or bis(acetyl)diaminopentane in a concentration-dependent fashion. Tetramethylurea, however, which is also a potent inducer of Friend leukemia cell differentiation, had no detectable effect on the rate of agglutination by this plant lectin. In contrast to other reports which have demonstrated no change in the number of concanavalin A receptors on the surface of DMSO-treated Friend erythroleukemia cells [8,9], we have found that treatment of these murine erythroleukemia cells with the polar solvent decreased the number of surface wheat germ agglutinin receptor sites.

## Materials and Methods

DMSO, purchased from Fischer Scientific Company (Pittsburg, PA), was distilled at 67°C under vacuum and stored frozen at −20°C in small vials which were thawed once immediately prior to each experiment. Dimethylformamide was obtained from Aldrich Chemical Company (Milwaukee, WI). Tetramethylurea was purchased from Sigma Chemical Company (St. Louis, MO). Concanavalin A, soybean agglutinin, Ricin, fucose-binding protein, and (G-$^3$H)-labeled wheat germ agglutinin (50 μCi/2.5 ml of phosphate-buffered saline, spec. act. 20 μCi/0.342 mg) were obtained from New England Nuclear Corporation (Boston, MA). Fluoresceneisothiocyanate wheat germ agglutinin and wheat germ agglutinin were purchased from Miles-Yeda, Ltd. (Rehovot, Israel). Bis(acetyl)diaminopentane was prepared by Dr. K.K. Bhargava in our laboratory. Benzidine hydrochloride was purchased from Mallinckrodt Incorporated (St. Louis, MO).

Murine erythroleukemia cells (clone 745) were donated by Dr. N. Gabelman (Mount Sinai School of Medicine, New York). Cells were maintained in suspension culture at 37°C in a 10% $CO_2$ humidified atmosphere in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 units/ml of penicillin and 100 μg/ml of streptomycin (GIBCO, Grand Island, NY).

*Induction experiments and determination of the proportion of hemoglobin-containing cells.* Induction of erythroid differentiation was carried out by inoculating exponentially growing cells at a level of $10^5$ cells/ml into medium containing either no drug, DMSO, or another inducer. Cells were then incubated at 37°C for six days. Cell numbers were determined using a Coulter Model ZBI Particle Counter; the instrument was calibrated for appropriate settings using polystyrene beads and hemocytometer readings. The degree of cell maturation was determined cytologically by the method of Orkin et al. [11]. Briefly, 100 μl of culture fluid were transferred into a Limbro microwell plate and mixed with 25 μl of benzidine peroxide stain. The proportion of differentiated cells (benzidine-positive cells) was ascertained by light microscopy counting at least 250 cells. Cell fragility was measured by the number of cells excluding trypan blue, as described previously [12].

*Assay of agglutination of erythroleukemia cells by plant lectins.* Agglutination of control and inducer-treated cells was carried out with a variety of plant lectins using previously reported methodology [13]. The rate of agglutination was measured over a time period of 20 min by determining the change in absorbancy at 546 nm of a cell suspension ($10^7$ cells/ml) incubated with a plant lectin. The concentration of lectin required to cause one-half of the

agglutination rate of cells ($K_a$) was determined from double-reciprocal plots (1/rate of agglutination vs. 1/concentration of lectin) as described by Vlodansky and Sachs [14].

*Assay for lectin binding to the cell surface.* The binding of radioactive $^3$H-labeled wheat germ agglutinin to murine erythroleukemia cells was measured by the method of Vlodansky and Sachs [14]. Friend leukemia cells were collected from cultures, washed three times with $Ca^{2+}$, $Mg^{2+}$-free Dulbecco's phosphate-buffered saline and resuspended at a concentration of $1.5 \cdot 10^6$ cells/ml. $^3$H-Labeled wheat germ agglutinin binding was measured by incubating 0.5 ml of cell suspension with 0.5 ml of phosphate-buffered saline containing various concentrations of lectin in the presence or absence of DMSO and the haptenic sugar *N*-acetyl-D-glucosamine. The reaction mixture was incubated at 22°C in a shaking water bath for 30 min. Incubation was terminated by the addition of 5 vol. of ice-cold phosphate-buffered saline, cellular pellets were collected by centrifugation at $650 \times g$ for 10 min, and the pellets were dissolved in 0.1 M NaOH; radioactivity present in each sample was determined in Aquasol using a Packard liquid scintillation spectrometer. Specific binding of wheat germ agglutinin was calculated by subtracting the amount of lectin bound to cells in the presence of 0.1 M *N*-acetyl-D-glucosamine (Sigma Chemical Company, St. Louis, MO) from the total bound lectin in the absence of the hapten sugar. The results were expressed as $\mu$g of $^3$H-labeled wheat germ agglutinin bound per $10^6$ cells.

*Assay for binding of fluorescene-labeled wheat germ agglutinin to murine erythroleukemia cells.* Fluorescene-labeled wheat germ agglutinin (2.0 mg/ml) was stored as a solution in 0.01 M phosphate-buffered saline (pH 7.5) at −20°C. Murine erythroleukemia cells were collected from cultures, washed three times with Dulbecco's phosphate-buffered saline and resuspended at a concentration of $10^6$ cells/ml. A portion of the cell suspension (0.5 ml) was mixed with 0.5 ml of phosphate-buffered saline containing 100 $\mu$g/ml of fluorescene-labeled wheat germ agglutinin and incubated at 4°C for 30 min. Incubation was terminated by dilution with cold phosphate-buffered saline and the cells were washed twice with phosphate-buffered saline containing 0.1 M *N*-acetyl-D-glucosamine. Smears of the cell suspension were prepared and fluorescence was determined with a Leitz microscope using transmitted ultraviolet light. About 90–95% of the cells were stained; the binding of fluorescene-labeled wheat germ agglutinin was specific, since cells incubated in the presence of 0.1 M *N*-acetyl-D-glucosamine did not show a fluorescent ring on the cell surface.

## Results

Wheat germ agglutinin, which recognizes the *N*-acetyl-D-glucosamine [14,15] and sialic acid residues on the cell surface, caused agglutination of Friend erythroleukemia cells that was characterized by a lag period followed by a relatively linear decrease in absorbance (Fig. 1). Wheat germ agglutinin-induced agglutination followed first order kinetics with respect to the concentration of lectin used, with the rate of agglutination reaching a maximum value at a level of 50 $\mu$g of wheat germ agglutinin/ml. That wheat germ agglutinin-induced agglutination involved the occupation of specific cellular binding sites was shown by the complete prevention of agglutination induced by 50 $\mu$g of wheat germ agglutinin/ml by 0.1 M *N*-acetyl-D-glucosamine, but not by the same concentration of either *N*-acetyl-D-galactosamine or $\alpha$-methyl mannoside, haptenic inhibitors of soybean agglutinin and concanavalin A, respectively. Murine erythroleukemia cells did not agglutinate well when other lectins with different surface carbohydrate receptor binding properties were used (Table I). The lectins employed included in addition to wheat germ agglutinin, concanavalin A, *Ricinus communis* lectin, soybean agglutinin and the fucose-binding protein. The limited agglutination produced by most of the plant lectins is presumably the result of either minimal binding of the lectin molecules or restricted lateral mobility of the involved receptor sites.

Direct evidence that both control and DMSO-treated cells bind wheat germ agglutinin on the cell surface was obtained using fluorescene-labeled wheat germ agglutinin. Control cells appeared to bind more fluorescene-labeled wheat germ agglutinin than those differentiated by exposure to 210 mM DMSO for six days, as determined by their fluorescence intensity. In addition, binding of fluorescene-labeled wheat

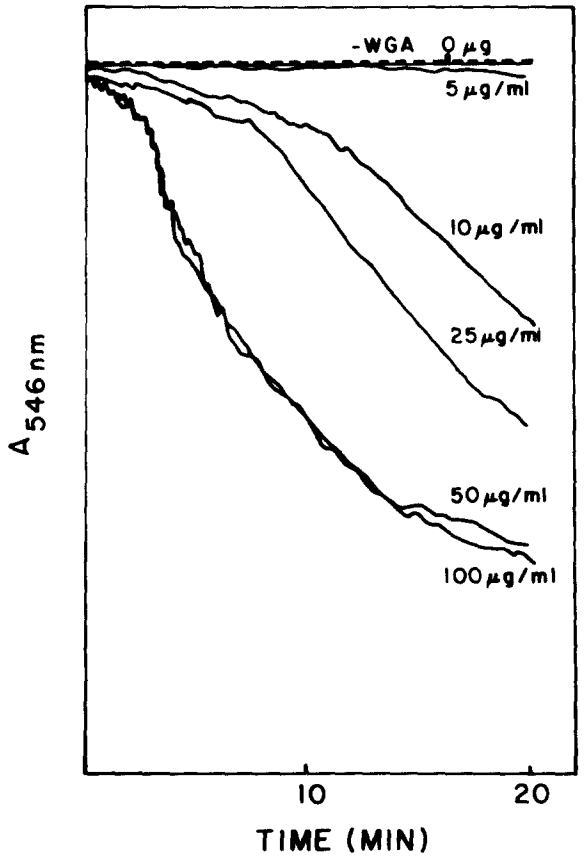


Fig. 1. Kinetics of wheat germ agglutinin-induced agglutination of murine erythroleukemia cells by different concentrations of lectin. Exponentially growing erythroleukemia cells were collected from the cultures, washed four times with $Ca^{2+}$, $Mg^{2+}$-free Dulbecco's phosphate-buffered saline (pH 7.4) and resuspended at a concentration of $10^7$ cells/ml. An aliquot of 1.0 ml of the cell suspension was placed into a cuvette and wheat germ agglutinin was added. The cell suspension was mixed and the rate of agglutination was measured over a time period of 20 min by determining the change in absorbancy at 546 nm. In control experiments, 0.1 M *N*-acetyl-D-glucosamine was added to the cell suspension prior to the addition of wheat germ agglutinin. The concentrations of wheat germ agglutinin employed were from 5 to 100 μg/ml; the levels used are given adjacent to the appropriate curve. The absorbance at 546 nm in the presence of 50 μg/ml wheat germ agglutinin and 0.1 M *N*-acetyl-D-glucosamine is shown (------). WGA, wheat germ agglutinin.

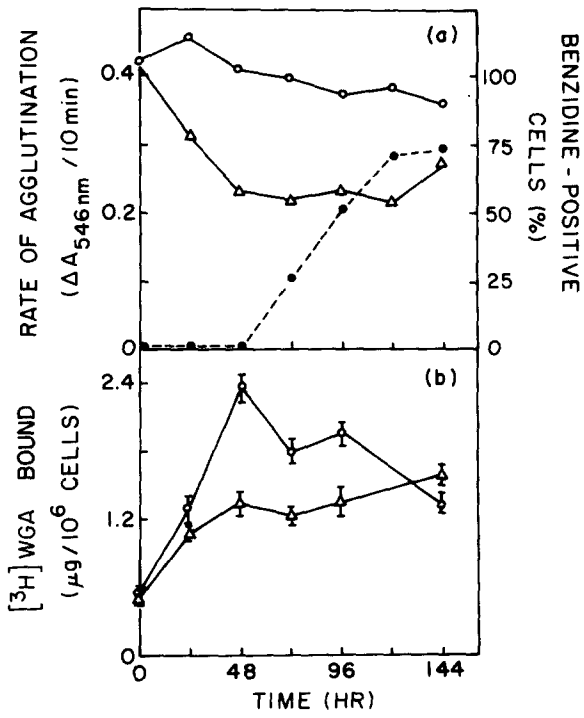


Fig. 2. Alterations in the rate of wheat germ agglutinin-induced agglutination and $^3$H-labeled wheat germ agglutinin ([$^3$H]WGA) binding to murine erythroleukemia cells during DMSO-induced differentiation. (a) Changes in the rate of cellular agglutination by 50 μg/ml of wheat germ agglutinin of control (○———○) and DMSO (210 mM) treated cells (△———△) at various times of incubation in Dulbecco's modified Eagle's medium. The proportion of benzidine-positive cells in DMSO-treated cultures (●·····●) was determined following exposure to 1.5% DMSO by the method of Orkin et al. [11]. (b) Changes in the specific binding of $^3$H-labeled wheat germ agglutinin of control (○———○) and DMSO (210 mM) treated cells (△———△) at various times of incubation; the bars refer to the S.E. from three separate experiments.

germ agglutinin to erythroleukemia surface membranes revealed dense areas in the form of patches or localized clusters (data not shown).

Significant differences in the rate of agglutination of murine leukemia cells by wheat germ agglutinin were detected during the process of DMSO-induced erythroid differentiation. The results shown in Fig. 2a indicate that the rate of wheat germ agglutinin-induced agglutination of untreated cells increased slightly at 24 h and decreased progressively thereafter to reach about 90% of the starting rate after 144 h of incubation. In contrast, the sensitivity of DMSO-treated Friend cells to agglutination by wheat germ agglutinin decreased by 25% within 24 h of exposure to the polar solvent, and reached a mini-

TABLE I

THE RATE OF AGGLUTINATION OF MURINE ERYTHROLEUKEMIA CELLS BY A VARIETY OF PLANT LECTINS

Exponentially growing Friend leukemia cells were collected from cultures by centrifugation at 350 × *g* for 10 min, washed four times with phosphate-buffered saline and resuspended at a concentration of $10^7$ cells/ml in $Ca^{2+}$, $Mg^{2+}$-free phosphate-buffered saline (pH 7.4). The rate of agglutination was determined spectrophotometrically using different concentrations of each plant lectin. The results are expressed as the change in absorbance at 546 nm of the cell suspension in 10 min. Ricin, *Ricinus communis* lectin.

| Lectin | Hapten sugar | Concentration (μg/ml) | Rate of agglutination ($A_{546nm}$/10 min) |
|---|---|---|---|
| None | – | – | 0.002 |
| Wheat germ agglutinin | *N*-Acetyl-D-glucosamine | 5 | 0.18 |
| | | 10 | 0.25 |
| | | 25 | 0.33 |
| | | 50 | 0.39 |
| | | 100 | 0.40 |
| Concanavalin A | α-Methyl mannoside | 10 | 0.008 |
| | | 25 | 0.012 |
| | | 50 | 0.028 |
| | | 100 | 0.032 |
| Ricin | D-Galactose | 25 | 0.005 |
| | | 50 | 0.005 |
| | | 100 | 0.005 |
| Soybean agglutinin | *N*-Acetyl-D-galactosamine | 25 | 0.008 |
| | | 75 | 0.008 |
| Fucose-binding protein | L-Fucose | 75 | 0.006 |
| | | 100 | 0.012 |
| | | 300 | 0.014 |

mum value (55% of the control rate) after 48 h of incubation. Thus, after a five-day period of exposure to DMSO which produced maximum differentiation, cells required larger amounts of wheat germ agglutinin ($K_a$ = 8.4 μg/ml) than control cells ($K_a$ = 6.5 μg/ml) to achieve the same agglutination rate. These findings indicate that DMSO-induced differentiation is accompanied by significant alterations in cell surface properties.

The changes in the rate of agglutination of the murine erythroleukemia cells by wheat germ agglutinin that occurred during the process of achieving a differentiated state could be attributable either to (a) an alteration in the concentration of lectin receptor sites or in their accessibility, (b) alterations in the plasma membrane fluid state induced by DMSO, or (c) other unknown factors. To determine whether changes in receptor sites for wheat germ agglutinin were produced by exposure of cells to DMSO, the binding of $^3$H-labeled wheat germ agglutinin was measured at various times of growth in the presence and absence of DMSO (Fig. 2b). The capacity to bind $^3$H-labeled wheat germ agglutinin increased markedly in untreated cells over a period of 48 h and then decreased progressively thereafter. $^3$H-Labeled wheat germ agglutinin binding also increased in cells exposed to DMSO but to a significantly lower degree, reaching a maximum value after 144 h of exposure to the polar solvent.

The effects of two additional potent inducers of differentiation, bis(acetyl)diaminopentane and tetramethylurea, as well as of several other membrane-active compounds were compared with those of DMSO on the wheat germ agglutinin-induced agglutination of murine erythroleukemia cells. Exposure to a concentration of 210 mM DMSO caused essentially immediate inhibition of wheat germ agglutinin-induced cell agglutination and an increase in the concentration of this solvent caused an even more pronounced decrease in the rate of agglutination

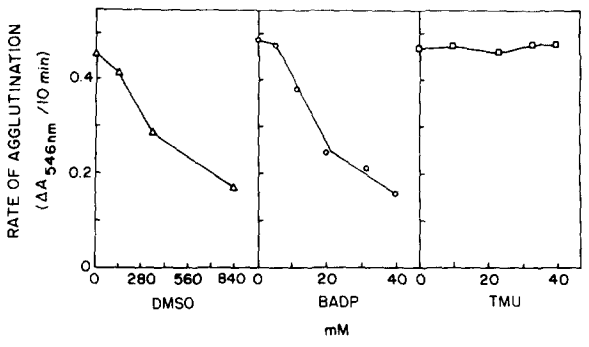


Fig. 3. The effects of DMSO, bis(acetyl)diaminopentane and tetramethylurea on the rate of agglutination of murine erythroleukemia cells by wheat germ agglutinin. Exponentially growing cells were collected from cultures by centrifugation at 350 × *g* for 5 min, washed four times in phosphate-buffered saline and resuspended at a level of $10^7$ cells/ml of $Ca^{2+}$, $Mg^{2+}$-free phosphate-buffered saline (pH 7.4). Agglutination by wheat germ agglutinin (50 μg/ml) was measured in the presence and absence of various concentrations of DMSO, bis(acetyl)diaminopentane and tetramethylurea. The data are expressed as changes in absorbance at 546 nm per 10 min of incubation at 22°C. DMSO (△); BADP, bis(acetyl)diaminopentane (○); TMU, tetramethylurea (□).

(Fig. 3). Similarly, bis(acetyl)diaminopentane interfered with wheat germ agglutinin-induced agglutination in a concentration-dependent manner. In contrast, however, tetramethylurea, a potent inducer of both DMSO-high and low-inducible murine erythroleukemia cells (unpublished observations), had no effect on the rate of agglutination by wheat germ agglutinin, even at the cytotoxic concentration of inducer of 40 mM. Inhibition of the rate of agglutination of murine leukemia cells by wheat germ agglutinin was also obtained with the inducer of differentiation dimethylformamide (data not shown). The degree of agglutination of erythroleukemia cells by wheat germ agglutinin was not altered by cytochalasin B or amphotericin B, two agents which interact with cell surface components but do not induce cell differentiation.

To ascertain whether the decreased binding of $^3$H-labeled wheat germ agglutinin by DMSO-treated cells relative to control untreated cells was attributable to a decrease in the number of receptors accessible to wheat germ agglutinin or to an altered affinity of the lectin for its receptor sites, the binding of radioactive wheat germ agglutinin in the presence of various concentrations of DMSO was subjected to Scatchard

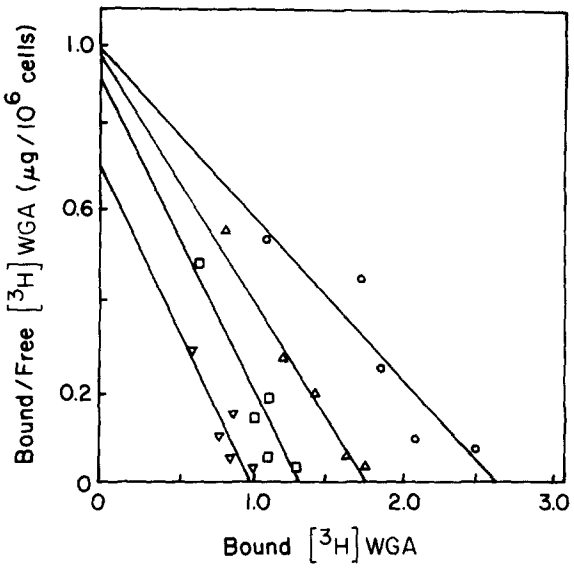


Fig. 4. Scatchard plot of the binding of $^3$H-labeled wheat germ agglutinin ([$^3$H]WGA) to murine erythroleukemia cells treated with DMSO. Exponentially growing erythroleukemia cells were collected from cultures 48 h after seeding, washed four times with $Ca^{2+}$, $Mg^{2+}$-free Dulbecco's phosphate-buffered saline (pH 7.4), resuspended at a concentration of $1.5 \cdot 10^6$ cells/ml and assayed for $^3$H-labeled wheat germ agglutinin binding as described in Materials and Methods. Specific binding of $^3$H-labeled wheat germ agglutinin was measured in murine erythroleukemia cells in the absence (○) and in the presence of various concentrations of DMSO (210 mM, △; 420 mM, □; 700 mM, ▽), as described in Materials and Methods. Least-square regression analysis was carried out to determine appropriate lines. Correlation coefficients were as follows: 0.902 (○); 0.989 (△); 0.930 (□); 0.887 (▽).

analysis [17]. The findings shown in Fig. 4 demonstrate that a level of 210 mM DMSO, the optimum concentration of the polar solvent for induction of erythroid maturation, caused a cellular change which resulted in a signficant decrease in the number of wheat germ agglutinin binding sites and a slight decrease in the affinity of this lectin for its cellular receptors.

## Discussion

The data presented in this report indicate that DMSO-induced cellular maturation of Friend erythroleukemia cells is accompanied by changes in surface membrane properties. The sensitivity of the murine erythroleukemia cells to agglutination by plant lectins

with different ligand specificities varied significantly. The murine erythroleukemia cell line employed (i.e., clone 745) was more susceptible to agglutination by wheat germ agglutinin than by a variety of other lectin molecules, allowing the use of wheat germ agglutinin as a probe for exploring surface changes occurring during the process of DMSO-induced erythroid maturation. This finding contrasted with those of other laboratories [7–9] which have reported different sensitivities to lectins in other erythroleukemia clones, presumably an expression of differences between these clones in surface membrane architecture.

A decrease in the rate of wheat germ agglutinin-induced agglutination of erythroleukemia cells was produced by exposure to DMSO; this change may be due either to altered accessibility of plant lectin receptor sites, to changes in the plasma membrane fluid state, as suggested by calorimetric studies with artificial liposome membranes treated with this polar solvent [18], or to other unknown factors. The finding of a decrease in agglutination of DMSO-treated cells by wheat germ agglutinin corresponded to a decrease in the amount of wheat germ agglutinin bound to these cells relative to that bound by control untreated cells; Scatchard analyses suggests that this phenomenon is attributable to a decrease in receptor accessibility resulting from either (a) alterations in the physical state of the cell surface or (b) disappearance of wheat germ agglutinin receptors during the differentiation process. Such changes are compatible with alterations in the fluid state of the plasma membrane of differentiated murine erythroleukemia cells, which probably occur in DMSO-treated cells due to marked decreases in the content of cholesterol relative to that of phospholipids [19,20]. Eisen et al. [8] and Hunt and Marshall [9] have reported no change in concanavalin A receptor density during the early portion of the differentiation process; although this appears to contrast with the reported changes in wheat germ agglutinin receptors in the present study, the latter authors [9] also demonstrated the formation and appearance of new concanavalin A receptors during the maturation process. Thus, the finding of no change in the total number of concanavalin A lectin receptor sites during erythroid differentiation is not indicative of a lack of major change in surface architecture.

The finding in the present study that changes in wheat germ agglutinin-induced agglutination and in $^{3}$H-labeled wheat germ agglutinin binding occurred within 24 h of incubation with DMSO, a time which corresponds to the period required for the cells to commit to differentiation [1,2,5], suggests that these changes may be indicative of early events in the maturation process. The concept gains support by the findings that bis(acetyl)diaminopentane and dimethylformamide caused similar alterations in agglutination properties. However, since the potent inducer of differentiation tetramethylurea [21] did not affect the rate of agglutination, either the effect of the inducers on the cell surface properties that are being measured using wheat germ agglutinin as a probe are not representative of obligatory initiative events in the differentiation process or, alternatively, there may be more than one mechanism involved in triggering the maturation process which depends upon the inducing agent employed.

Changes in the cell surface of DMSO-treated cells as monitored by both plant lectin agglutination and plant lectin binding appear to be consistent with the finding of (a) differences in other surface characteristics of DMSO-induced erythroleukemia cells, such as surface charge [22,23] and permeability [24]; (b) a marked decrease in the cholesterol content of differentiated cells [19]; (c) a change in the rate of incorporation of precursors of glycoprotein and phospholipid in DMSO-treated cells [19,25]; and (d) the disappearance of microvilli from the surface of DMSO- or bis(acetyl)diaminopentane-induced cells [9]. Since components such as glycoproteins and cholesterol represent major determinants of the physical state of the cell surface [26–28], and are involved in regulating the rate of cell agglutination [14,26], it is clear that DMSO causes a series of events at the level of the plasma membrane.

The precise mechanism by which interactions between inducers and murine erythroleukemia cells lead to the initiation of a developmental program, remains to be understood. However, a correlation between cryoprotective ability [21], polarity [29], and the capacity to induce maturation for the majority of the chemical inducers [1] suggest that interactions of these compounds with cell surface components may be fundamental in triggering the differentiation of Friend cells. Additional evidence

which suggests that induction of erythroid differentiation of Friend leukemia cells is associated with cell surface phenomena includes: (a) a large majority of chemical inducers are lipophilic [21,29]; (b) short-chain fatty acids can initiate differentiation [30]; (c) DMSO and other inducers affect the phospholipid transition phase temperature of unilamellar liposomes [18]; (d) ouabain, an inhibitor of the membrane-bound enzyme ($Na^+ + K^+$)-ATPase, is a potent inducer of Friend leukemia differentiation [31]; (e) local anesthetics such as procaine inhibit DMSO-induced differentiation [32,33] and bis(acetyl)-diaminopentane localizes largely in the plasma membrane [34]. The membrane changes observed during differentiation of murine leukemia cells are consistent with the concept that DMSO or other inducers may alter the fluid state of the plasma membrane and in turn affect the mobility of proteins and their degree of exposure [35]. However, it is still unclear whether surface properties described here and elsewhere [7–9] are directly involved in the initiation of erythrodifferentiation, or are the result of the maturation process itself in a manner analogous to the appearance of membrane proteins such as transferrin receptors [36], spectrin [37], erythrocytic antigens [38], and a transmembrane glycoprotein designated band 3 [39].

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