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THE INHIBITORY EFFECT OF SOME LECTINS ON THE DIFFERENTIATION OF FRIEND ERYTHROLEUKEMIA CELLS

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Summary: Growth and differentiation of Friend cells can be inhibited by treatment with concanavalin A and wheat-germ agglutinin. This inhibition is specific for cells that are in the early stages of the differentiation process (24-48 h after the addition of dimethyl sulfoxide) and is reversible upon treatment with the sugars competitive for these lectins. These results suggest a regulatory role for some plasma membrane-bound glycoproteins early in the differentiation of Friend erythroleukemia cells induced by dimethyl sulfoxide.

Friend virus-transformed erythroleukemia cells form a valuable system for the study of erythroid differentiation. When induced by dimethyl sulfoxide (DMSO) or other agents they undergo a series of changes also seen during normal erythropoiesis such as terminal cell division and accumulation of hemoglobin (1). In studies to elucidate the mechanisms by which this process may be governed many changes have been described to take place in the period following the addition of the inducing agent (1). These changes include alterations in the synthesis and/or glycosylation of some plasma membrane glycoproteins (2,3). Some of these changes, as revealed by labelling experiments or by lectin binding and agglutinability, are most conspicuous around the the time at which the maximal number of cells becomes irreversibly committed to terminal differentiation (3,4,5). These observations led us and others to suggest that alterations in the properties of some membrane glycoproteins may be closely associated with the differentiation process itself (4,5,6). The finding that specific inhibitors of protein glycosylation

Abbreviations: DMSO, dimethyl sulfoxide; PNA, peanut agglutinin; SBA, soybean agglutinin; Con A, concanavalin A; WGA, wheat-germ agglutinin; Hb, hemoglobin.

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inhibit DMSO-induced differentiation by interfering with the process by which the cells become committed, substantiated this suggestion (7). In this study we describe the use of some lectins to expand our investigations on the possible role of plasma membrane-bound glycoproteins in the DMSO-induced differentiation of Friend cells.

MATERIALS AND METHODS

Friend cells (cell line GM-86, clone 745) were purchased from the Institute for Medical Research (Camden, N.J.). Peanut agglutinin (PNA) and soybean agglutinin (SBA) were obtained from Boehringer, concanavalin A (Con A) was from Sigma and wheat-germ agglutinin (WGA) was purchased from Pharmacia. N-[acetyl-^{3}H] concanavalin A (30 - 90 Ci/mmol) was purchased from Amersham. All other reagents were analytical grade.
Cells were cultured and induced to differentiate with dimethyl sulfoxide (DMSO, 1.5%, v/v, Merck) as described before (7). Differentiation was measured by determining the hemoglobin content of 1.10^7 cells at 410 nm (8). Cell viability was determined by dye exclusion after staining in 0.01% trypan blue. Concentrated solutions of the lectins were sterilized by passing through a Millipore filter (0.25 µm).
Lectin treatment of the cells was performed under sterile conditions by incubating 1.10^7 washed cells with a lectin (250 µg/ml) in a final volume of 1 ml of buffer (Tris 50 mM, NaCl 0.15 M, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM, $MnCl_2$ 1 mM, pH 7.6) for 15 minutes at 37° C. After the incubation cells were washed twice with the same buffer and reseeded into culture medium of the same composition and at the same cell density as prior to the incubation. Control cells underwent the same treatment with the omission of the lectin in the incubation mixture.

RESULTS AND DISCUSSION

When Friend cells were seeded into medium containing DMSO, maximal differentiation was seen after 96 h as measured by the hemoglobin content of the cells, which is in agreement with earlier observations (7,9). Growing the cells in the continuous presence of various lectins (SBA, Con A or WGA) in a concentration up to 1 µg/ml -which is sufficient for maximal agglutination (4,5)- for 96 h with or without DMSO had no effect on cell growth or differentiation, nor had the addition of these lectins at different times after the start of the culture (data not shown). Peanut agglutinin in the same concentration, however, seemingly inhibited the differentiation but this was shown to be caused by a toxic effect of this lectin on fully differentiated cells; when differentiated cells were washed and incubated with PNA (1 µg/ml) for 6 h, about 60% of the hemoglobin content of the cells was found in the supernatant, corresponding with 80%

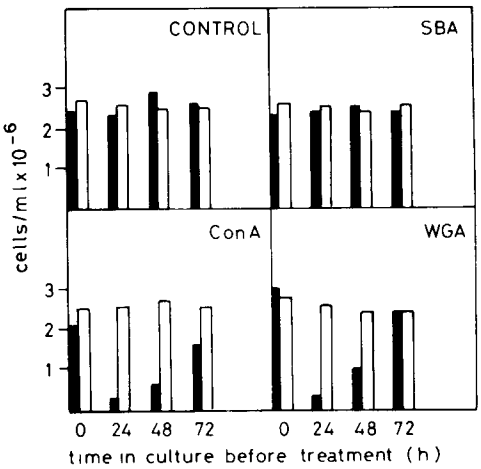

Figure 1: "The effect of some lectins on the growth of Friend cells in the presence and absence of DMSO".

Cells were seeded into medium with or without DMSO at a starting density of 1.10^5 cells/ml. After the indicated periods in culture cells were treated with or without a lectin (250 µg/ml) for 15 minutes, washed and reseeded into fresh original medium. After a total time in culture of 96 h cell density and viability were determined (for details see Materials and Methods).

Open bars, cells cultured without DMSO; black bars, cells cultured with DMSO.

non-viable cells as judged by trypan blue exclusion. The other lectins had no effect on cell viability. This effect of PNA may very well be correlated with the appearance of a specific receptor for this lectin in differentiated cells as revealed by the binding of iodinated PNA to a 150 K glycoprotein after gel electrophoresis of a plasma membrane fraction (10).

As the conditions of these experiments may obscure a possible effect of some lectins on the differentiation, *e.g.* because preferential binding of lectins to serum components or degradation during a prolonged incubation time may occur, we followed the elegant experimental design of Azhar and Menon (11). Cells were seeded into medium with or without DMSO. At various times after the start of the culture cells were spun down and incubated with a lectin in a concentration (250 µg/ml) very much higher than used in the experiment described above. After 15 min the cells were washed to remove unbound lectin and reseeded into fresh medium of the same composition as in which they had been cultured before this treatment (for

Table 1: "The effect of some lectins on hemoglobin synthesis of differentiating Friend cells".

time in culture before treatment (h)	Hb ($A_{410}/1.10^7$ cells)			
	lectin			
	none	SBA	Con A	WGA
0	0.24 ± 0.02	0.22 ± 0.02	0.20 ± 0.02	0.23 ± 0.02
24	0.25 ± 0.02	0.24 ± 0.02	0.03 ± 0.01	0.04 ± 0.01
48	0.23 ± 0.02	0.23 ± 0.01	0.08 ± 0.01	0.13 ± 0.01
72	0.26 ± 0.01	0.24 ± 0.02	0.15 ± 0.01	0.20 ± 0.02

Cells were seeded into medium with DMSO at a starting density of 1.10^5 cells /ml. After various periods in culture cells were treated for 15 minutes with or without a lectin (250 µg/ml) washed and reseeded into fresh medium with DMSO. After a total time in culture of 96 h cell density, viability and hemoglobin content were determined (for details see Materials and Methods). The hemoglobin content is expressed as the absorption at 410 nm of 1.10^7 cells after lysis. All values are the mean of four experiments (± S.D.).

details see Materials and Methods). After a total time in culture of 96 h cell density , viability and hemoglobin content were determined. As judged by trypan blue exclusion, in all experiments more than 95% of the cells remained viable, both just after the treatment and after a total time in culture of 96 h.

This treatment with the various lectins tested had no effect on the growth of cells cultured in the absence of DMSO (Fig. 1). However, when cells were treated after different periods in culture with DMSO, Con A and WGA inhibited cell growth markedly, while SBA (and PNA, data not shown) had no such effect. Furthermore, maximal inhibition occurred in the case of cells that had first been cultured with DMSO for 24 h . Treatment of the cells with Con A or WGA before the start of the culture or after longer periods of growth than 24 h in the presence of DMSO resulted in a much lower degree of inhibition (Fig. 1). Inhibition of cell growth ran parallel with inhibition of differentiation as judged from the hemoglobin content of the cells (Table 1).

When differentiating cells were incubated with Con A or WGA in the simultaneous presence of their competitive sugars (α-methyl-D-mannoside

Table 2: "The effect of increasing concentrations of concanavalin A and wheat-germ agglutinin on cell growth and hemoglobin synthesis of differentiating Friend cells".

addition	concentration (μg/ml)	cells/ml x 10^{-6}	Hb ($A_{410}/1.10^7$ cells)
Con A	0	2.2	0.26
	10	1.0	0.14
	25	0.5	0.07
	50	0.4	0.05
	100	0.3	0.04
Con A (250 μg/ml) + α-methyl-D-mannoside (0.2 M)		2.3	0.27
WGA	0	2.3	0.25
	10	2.2	0.25
	25	1.0	0.13
	50	0.8	0.06
	100	0.6	0.05
	250	0.3	0.04
WGA (250 μg/ml) + N-acetyl-D-glucosamine (0.2 M)		2.4	0.24

Cells were seeded into medium with DMSO at a density of 1.10^5 cells/ml. After 24 h cells were treated with lectins (concentrations as indicated), reseeded into medium with DMSO and cultured for another 72 h. After this time cell density and hemoglobin content were determined as described in the legend to Table 1. The data of one representative experiment are presented.

and N-acetyl-D-glucosamine respectively) no inhibition of cell growth or hemoglobin synthesis was observed, suggesting that inhibition is related to specific binding of these lectins to the cell surface (Table 2). It was further shown that the degree of inhibition was dependent upon the concentration of the lectin (Table 2). Moreover, this inhibition is reversible: when lectin-inhibited cells, treated with Con A or WGA after 24 h in culture with DMSO and kept in this medium for 72 h, were incubated for 15 min with the competitive sugars and reseeded into fresh medium with DMSO, cell growth and differentiation were completely restored after 72 h of subsequent culture.

When cells that had been cultured with or without DMSO for 24 h were treated with a mixture of Con A and ^{3}H-Con A, undifferentiated and differentiated cells bound radioactivity to the same extend to begin with Table 3, first line), which is in agreement with earlier observations (4). In the case of undifferentiated cells this binding has no effect upon

Table 3: "The binding of ^{3}H-Con A to lectin treated Friend cells".

time in culture after treatment (h)	cells/ml x 10^{-6}		cpm/10^{7} cells		% radioactivity released by 0.2 M αMM	
	+	-	+	-	+	-
0	0.2	0.3	20 000	19 000	80	80
24	0.2	0.7	19 000	10 000	85	90
72	0.3	2.5	17 000	2 000	90	90
(1)	(2)		(3)		(4)	

Cells were seeded into medium with or without DMSO (1.10^{5} cells/ml). After 24 h cells were treated with Con A (250 µg/ml) containing 0.5 µCi ^{3}H-Con A, washed and reseeded into medium with DMSO. At the times indicated cells were washed, incubated with α-methyl-D-mannoside (αMM) for 15 minutes at 37° C and the radioactivity in cells and supernatant was determined. The data of one representative experiment are presented.

subsequent cell growth (Table 3, column 1; see also Fig. 1). Most of the Con A remains bound at the outside of the cells and can be released by α-methyl-D-mannoside (Table 3, column 4).

From these experiments we may conclude that the binding of Con A and WGA inhibits cell growth and differentiation of Friend cells cultured in the presence of DMSO, but not the growth of non-differentiating cells. The simplest explanation of this difference would be to assume that in the former case Con A and WGA, which have a high affinity towards glycoproteins containing N-linked carbohydrate chains, block one or more specific membrane glycoproteins that have to be accessible in order for the cells to grow and differentiate. The concentration of such (a) glycoprotein(s) would reach its peak about 24 h after adding DMSO, but would be much lower or zero in non-differentiating cells.

Previous studies have shown that inhibition of N-linked glycosylation also inhibits differentiation early (24-48 h) after the addition of DMSO (7). This is also the time at which changes in lectin agglutinability (4, 5) and in the synthesis of membrane glycoproteins (2, 3) are most conspicuous and the cells become irreversibly committed to terminal differentiation. Viewed in this light, the present results support the suggestion that plasma membrane-bound glycoproteins play

an -as yet unknown- role in the DMSO-induced differentiation of Friend erythroleukemia cells.

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