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Binding of Wheat Germ Agglutinin by HeLa Cells: Effects of Prednisolone (38011)

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(Introduced by P. Morgan)

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Glucocorticoids induce changes in several properties associated with the cell surface of cultured epithelioid cells (1–3). Addition of prednisolone to HeLa cells causes a reduction in cell multiplication, a considerable increase in cell size, and a pattern of culture growth that resembles density-induced inhibition (4). In such steroid-treated cultures, the formation of multilayered cell aggregates is reduced and the pattern of the cell layer is more uniform than in corresponding controls. Moreover, the survival of cells treated by steroids is improved if the culture is subjected to nutritional or mechanical stress (5–7). The mechanism of glucocorticoid effects on heteroploid cells is poorly elucidated; the only knowledge about the altered chemical composition of the cell periphery is the reported increase in the sialic acid content (8,9). Recently, an observation was made in our laboratory (10) of a decreased shedding of sialopeptides from the surface of prednisolone-treated cells. This finding was consistent with previous work showing that increased agglutination of prednisolone-treated HeLa cells by wheat germ agglutinin (WGA) was abolished by a pretreatment with neuraminidase (11). The requirement for sialic acid in agglutination of L1210 ascites by WGA was demonstrated earlier by Burger and Goldberg (12).

The results reported here show that HeLa cells grown in the presence of 0.5 μg/ml prednisolone bind more WGA than cells grown for the same length of time in the absence of this steroid. To demonstrate this effect, we used optical observations as well as a new technique making use of a platelet aggregometer with rabbit erythrocytes as an indicator system.

Materials and Methods. Cells and medium. Two strains of HeLa S3 cells were used in this study. Their cultivation and properties were described in detail in an earlier paper (4). Cells were subcultured by using 0.05% trypsin made up in Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution (BSS). Cultures were monitored regularly for possible mycoplasmal contamination and were found to be negative.

Wheat germ lipase agglutinin. The agglutinin was prepared from wheat germ lipase (Sigma Chemical Company, St. Louis, MO) according to the method of Burger and Goldberg (12). Five grams of wheat germ lipase was suspended in 250 ml of distilled water and ground in a mortar; the suspension was further treated in an Elvehjem–Potter glass homogenizer. The homogenized preparation was heated for 15 min at 63° and was centrifuged at 170*g* for 15 min. The supernate was decanted and 57 g of $(NH_4)_2SO_4$ was added slowly at 0°. The material was centrifuged at 10,000*g* for 10 min. The supernate was discarded; the precipitate was dissolved in 7 ml of distilled water and dialyzed at 4° against 500 vol of distilled water for 24 hr. The dialyzed material containing 52.4 mg of protein/ml was kept frozen.

Agglutination assays. For agglutination assays, 3-day-old cells were detached from the glass surface with disodium ethylenediaminetetraacetate (EDTA) prepared as a 0.02% solution in Ca^{2+}- and Mg^{2+}-free

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TABLE I. Effect of Prednisolone in the Culture Medium and *N*-Acetylneuraminic Acid on Agglutination of HeLa S3G Cells by WGA.[a]

Reciprocal dilution of standard WGA	Prednisolone during growth (0.5 μg/ml)	Agglutinating activity	
		WGA	WGA plus NANA (4×10^{-3} *M*)
20	Absent	4+	4+
80	Absent	2+	4+
160	Absent	1+	2+
320	Absent	0	2+
20	Present	4+	4+
80	Present	3+	4+
160	Present	2+	4+
320	Present	1+	2+

[a] Conditions: The cells were grown for 3 days either in the presence or absence of prednisolone. They were detached from glass by EDTA (0.02% solution in 0.85% NaCl solution), washed repeatedly, and adjusted to 5×10^5 cells/ml. Each assay mixture contained 1.0 ml of cell suspension, with or without NANA, and 50 μl of the standard preparation of the purified WGA.

Hanks' BSS. For the optical assay, the detached cells were diluted to 5×10^5 cells/ml. One milliliter of suspension, with or without *N*-acetylneuraminic acid (NANA), was dispensed with a Cornwall syringe into each well of disposable plastic trays; 50 μl of an appropriate dilution of the standard preparation of WGA was then added to each well. The trays were incubated for 15 min at room temperature and were examined microscopically with a low-power objective. The agglutination was evaluated according to an arbitrary scale; a 4+ rating represented total agglutination of cells. This method was satisfactory provided enough replicate samples were included in each assay. It was less suitable for evaluating small differences in agglutination.

Thus an alternate procedure utilizing a modified platelet aggregometer (Chrono-Log Corporation, Broomall, PA) was devised. The instrument allows measurement of shifts in light transmission during platelet aggregation. The cuvet which contains a small magnetic stirrer to keep the cells in suspension is thermostatically controlled at 37°. The shifts in transmission are recorded on a Bausch and Lomb VOM7 strip chart recorder. A similar instrument ("Fragilograph," Kalmedic Instruments, Inc., 425 Park Avenue, New York, NY) provided with a platelet adaptor may be suitable for such studies.[1] In our initial experiments with the aggregometer, agglutination of HeLa cells was followed directly by adding WGA to the cuvet containing a suspension of HeLa cells, but the resulting cell clumps were too large, causing drifting and lack of reproducibility of aggregation plots. An indirect method using rabbit erythrocytes proved to be successful. The procedure consisted of adsorbing a standard solution of WGA with suspensions of HeLa cells adjusted to the same cell number. After adsorption, the supernate was tested for its ability to agglutinate rabbit erythrocytes. The erythrocytes were readily agglutinated by WGA forming uniform clumps that could be traced in the aggregometer.

Results and Discussion. Increased agglutination of HeLa cells grown in the presence of prednisolone is shown in Table I. Particularly noteworthy was the observation that NANA, when added to the reaction mixture that included cells and WGA, had a potentiating effect on agglutination and reduced simultaneously the difference between the agglutinability of prednisolone-treated and control cells. A lack of haptenlike activity of NANA in the WGA agglutinating system was reported (12), but the increase in agglutination after adding

[1] Dr. Perry Morgan, personal communication.

TABLE II. Effects of *N*-Acetyl-D-Glucosamine on the Agglutination of HeLa Cells by WGA.[a]

Cell strain	Reciprocal dilution of standard WGA	Agglutinating activity		
		GlcNAc added (1×10^{-3} *M*)		
		Before adding WGA	After adding WGA	Control (no GlcNAc)
HeLa S3G	20	2+	4+	4+
	80	0	2+	2+
	160	0	1+	1+
	320	0	0	0
HeLa S3K	20	2+	4+	4+
	80	0	2+	2+
	160	0	1+	1+
	320	0	0	0

[a] Conditions: Procedure was the same as described in Table I. The Glc*N*Ac was added to the cells either 30 min before WGA or 30 min after WGA.

NANA to the reaction system is a new observation. Perhaps, as suggested recently by Greenaway and LeVine (13), interaction of NANA with the lectin brings about optimal orientation of the WGA molecule during the agglutination reaction. The effects of *N*-acetyl-D-glucosamine (Glc*N*Ac) on agglutination were examined in both the microscopic assay with HeLa cells and in the aggregometer assay using rabbit erythrocytes. When Glc*N*Ac was added to HeLa cells before addition of WGA, it prevented agglutination (Table II); however, in contrast to the results of Burger and Goldberg (12), HeLa cells could not be disaggregated by Glc*N*Ac after agglutination had taken place. Possibly, the higher content of sialic acid in glass-grown HeLa cells as opposed to the suspension-grown L1210 line is responsible for the difference.

The rabbit erythrocytes were not agglutinated in the aggregometer in the presence of Glc*N*Ac [Fig. 1 (I)], indicating that this system showed specificity for WGA and could be used for detecting differences in the amount of WGA bound by HeLa cells. Figure 1 (II and III) shows superimposed plots representing agglutination of rabbit erythrocytes by the residual WGA after its adsorption by prednisolone-treated and nontreated cells together with the nonadsorbed agglutinin of the same dilution. It is evident that with both HeLa strains, the supernate from steroid-treated cells showed reduced agglutinating potency toward rabbit erythrocytes. These agglutination assays were repeated over 30 times with cells adjusted to various densities and with various agglutinin titers. The results were consistent indicating without exception that suspensions of steroid-treated cells were more effective in reducing the titer of WGA in the supernatant medium than the control cells.

At this time no method is available for the quantitative recovery of the adsorbed WGA; therefore, one cannot be certain whether reduction in agglutinability of rabbit erythrocytes results from the binding of agglutinin to HeLa cells or is caused by some other mechanism of inactivation of WGA. Moreover, there is no indication at the present time whether the assumed increased binding of WGA by the prednisolone-treated cells reflects an increase in the number of specific sites for binding WGA or is a result of altered topography of the cell's surface. A recently developed electron microscopic method for demonstrating sialic acid residues at the cell surface (Boyd, Melnykovych, and Fiskin, in preparation) might be helpful in answering some of these questions.

Experiments are currently in progress to determine whether the sialopeptides that are

being released from HeLa cells into the medium can interact with WGA and prevent its binding to the intact cells. This is particularly important in connection with the reported isolation and identification of WGA receptors (14, 15). The aggregometer assay may permit the expression of such data in a more objective manner than the presently available microscopic assays.

Summary. A new technique utilizing a platelet aggregometer was devised for evaluating agglutination of tissue culture cells by wheat germ lipase agglutinin (WGA). The hypothesis that HeLa cells grown in the presence of glucocorticoids accumulate sialopeptides at the cell surface was examined by subjecting such cells to agglutination by WGA. The steroid-treated cells were found to bind more agglutinin at the cell surface and were agglutinated more strongly than the controls.

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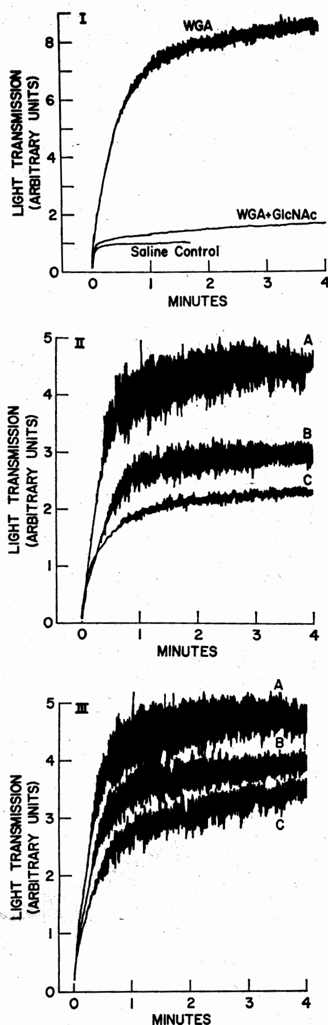

FIG. 1. (I) Inhibition of WGA-induced agglutination of rabbit erythrocytes by *N*-acetyl-D-glucosamine (Glc*N*Ac). Freshly washed rabbit erythrocytes were adjusted to a 0.5% suspension in 0.85% NaCl solution. A 0.4-ml portion was placed into the aggregometer cuvet and equilibrated with stirring for 5 min. A solution of WGA (50 μl of 1:40 dilution of the standard preparation) was then injected rapidly with an Eppendorff pipet. The Glc*N*Ac was added before WGA after adsorption of a standard WGA solu- individual tracings were superimposed for the sake of comparison. (II and III) Superimposed tracings of aggregation of rabbit erythrocytes by WGA at a final concentration of 10^{-3} *M*. The tion by HeLa cells. HeLa cells were adjusted to a density of 1×10^6 cells/ml and incubated with the WGA solution at 25° for 30 min. The cells were then centrifuged and the supernate was tested for agglutination of rabbit erythrocytes as described under (I). I = HeLa S3G, II = HeLa S3K; A = nonadsorbed control WGA, B = WGA adsorbed by cells grown without prednisolone, C = WGA adsorbed by cells grown for 3 days with 0.5 μg/ml prednisolone.

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