CHANGES IN THE BINDING OF CONCANAVALIN A AND WHEAT GERM AGGLUTININ TO HUMAN LYMPHOCYTES DURING IN VITRO TRANSFORMATION

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SUMMARY. Concanavalin A binds to human circulating lymphocytes in a complex manner suggesting the presence of multiple binding sites. Saturation of one or more of these binding sites is observed at concentrations of concanavalin A which induce blast transformation in lymphocytes. In contrast, only one saturable binding site is observed for wheat germ agglutinin. During in vitro transformation, the amount of concanavalin A which can be bound by lymphocytes increases, whereas the amount of wheat germ agglutinin which can be bound remains unchanged. Since the size increases during transformation, there must be a fall in the density of surface receptors for wheat germ agglutinin whereas the density of concanavalin A receptors remains unchanged.

The plant lectin, concanavalin A (Con A), can induce blast transformation in human lymphocytes (1). Recent studies in our laboratory have demonstrated the de novo emergence of insulin receptors on the surface of human lymphocytes during Con A mediated blast transformation (2). We have also observed that Con A and another plant lectin, wheat germ agglutinin (WGA), have potent insulin-like activity in fat cells (3) and that they can interact directly with insulin receptor structures (3, 4). The striking appearance of insulin receptors on the surface of lymphocytes during the process of in vitro blast transformation prompted us to examine simultaneous changes in the binding of Con A and WGA to the cell surface.

MATERIALS AND METHODS. Con A (3-X recrystallized) was obtained from Miles-Yeda. ^{125}I-Con A (18 to 25 Ci per g) was prepared by the chloramine-T method (5, 6) and purified by adsorption to Sephadex G-100 (5). The specific activity of ^{125}I-Con A was reduced by addition of native Con A for concentrations greater than 0.1 μg per ml. ^{125}I-WGA (2 Ci per g) and native WGA were purified by affinity chromatography on ovomucoid-agarose (5); the radioactive lectin was diluted with native WGA for concentrations greater than 0.67 μg per ml.

Human permanent lymphocyte strain RPMI 6237 was obtained from Associated Biomedic Systems. Human circulating lymphocytes were isolated from fresh heparinized Type O Rh$^+$ blood after filtration through nylon wool columns 4 x 8 cm (LP-1 Leuko-Pak leukocyte filters, Fenwal Laboratories, Morton Grove, Ill.). Erythrocytes were allowed to settle by gravity at 37° and the lymphocytes (95 to 98% pure) were withdrawn from the cell-rich plasma, washed three times with Medium 199 and harvested by centrifugation (150 g x 15 minutes each wash). The cells were cultured as described earlier (2).

Before measuring binding, lymphocytes were incubated with 50 mM α-methyl-D-mannopyranoside for 40 minutes at 37° to dissociate the Con A that had been bound during the culture period. The sugar was removed with two washings of Hanks' buffer containing 0.1% (w/v) albumin. Residual erythrocytes were lysed by brief hypotonic shock in Hanks' buffer lacking sodium chloride and glucose, followed by a 20-fold dilution with isotonic Hanks' buffer containing 0.1% albumin. Lymphocytes were recovered by centrifugation at 100 x g for 20 minutes, allowing red cell ghosts to remain in the supernatant.

To measure lectin binding, lymphocytes (6 to 7 x 10^5 per ml for Con A, 2 x 10^6 per ml for WGA) were suspended in Hanks' buffer containing 0.1% albumin and distributed in sets of four 0.2 ml aliquots. Two samples of each set were preincubated with native lectin (1 mg per ml for Con A, 0.4 mg per ml for WGA) for 10 minutes. The iodolectin was added to all the samples, each set of four corresponding to one concentration of ^{125}I-lectin. After incubating for 30 minutes at 24°, the cells were collected by membrane filtration and washed with 10 ml of ice-cold Krebs-Ringer bicarbonate buffer containing 0.1% bovine albumin (5). The binding of lectin in duplicate samples was corrected for the amount bound in the presence of the native lectin (5).

RESULTS. Binding of Con A - Con A binding to lymphocytes is not a simple function of lectin concentration (Fig. 1). Reproducibly, there is an indication of three

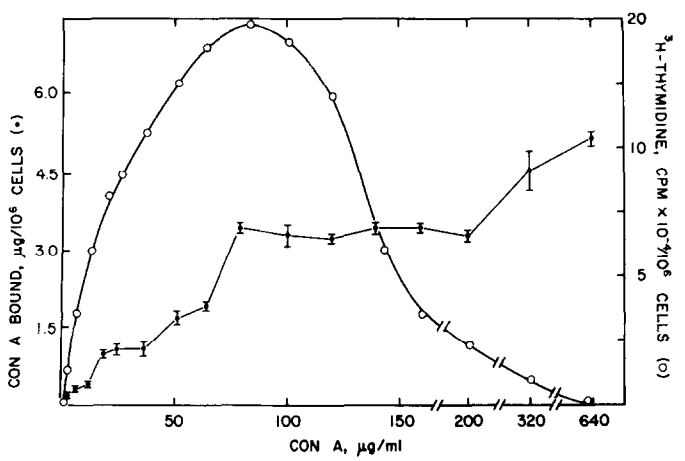

Fig. 1 - Mitogenic activity and binding of Con A to lymphocytes as a function of lectin concentration. The amount of Con A bound represents the mean ± s.e. of three experiments with untransformed cells. The mitogenic activity of Con A in cpm x 10^{-4} per 10^6 cells was determined (2) by pulse-labelling for 3 hours with 1 µCi per ml of [^{3}H]-thymidine at 48 hours after the addition of Con A to triplicate 1 ml samples of lymphocytes (10^6 per ml) in Medium 199 containing 10% (v/v) fresh human serum.

saturation plateaus which occur at about 15, 35 and 80 μg of Con A per ml. A similarly complex binding curve, suggesting the existence of multiple binding sites for Con A, is observed in both transformed and untransformed lymphocytes, and in the presumably homogeneous permanent lymphocyte strain RPMI 6237. Control experiments with RPMI 6237 lymphocytes show that neither incubation in 50 mM α-methyl-D-mannopyranoside nor treatment with hypotonic Hanks' solution affect the subsequent binding of the lectin to the cells. Con A binding to lymphocytes is not affected by the presence of glucose (5.6 mM) in Hanks' buffer.

While the overall shape of the binding curve is identical for both transformed and untransformed lymphocytes, it is found that at all concentrations of Con A transformed cells bind more lectin than do untransformed cells. Detail of a linear portion of the Con A binding curve in the concentration range 0 to 1 μg per ml is presented in Fig. 2 (lower). During transformation, the amount of Con

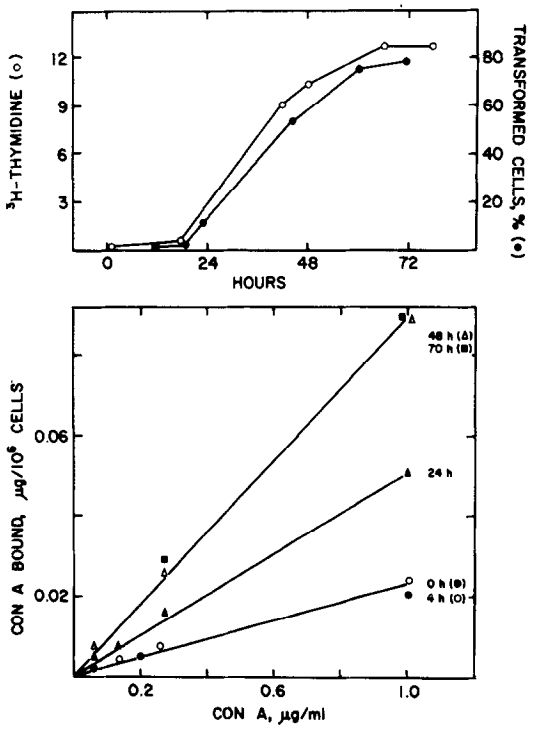

Fig. 2 - Upper: Time course of Con A-induced transformation of human circulating lymphocytes in vitro. The % of transformed cells (microscopically) and the incorporation of [^{3}H]-thymidine into DNA (cpm x 10^{-4} per 10^6 cells) were determined as described previously (2). Lower: Binding of ^{125}I-Con A to lymphocytes at various times during transformation by Con A. Data is presented only for the low range of lectin concentration (see Fig. 1), where the binding is related linearly to the medium concentration of lectin. Similar differences in Con A binding occur at higher concentrations where the binding curve is more complex.

A bound begins to rise between 18 and 24 hours and reaches a maximum at 48 hours. The time course of this increase in binding can be compared with the appearance of transformed cells, detected by changes in DNA synthesis, and by the dramatic increase in the proportion of enlarged cells (Fig. 2). In six separate experiments, the binding of Con A increased about 2- to 4-fold over the entire range of lectin concentration. Concommitantly, the measured diameter of the cells increased by a factor of 1.8, corresponding to a three-fold increase in mean cell surface area.

Binding of WGA - In contrast to the results with Con A, only one saturable binding site is discernable for WGA (Fig. 3). Furthermore, no difference can be

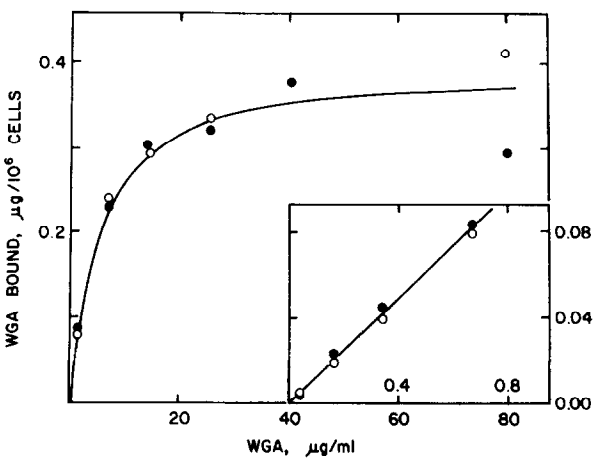

Fig. 3 - Binding of ^{125}I-WGA to untransformed (o) and transformed (•) lymphocytes as a function of lectin concentration.

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detected between transformed and untransformed cells either at the highest or lowest concentrations of lectin examined (insert, Fig. 3). The exact quantity of WGA bound to lymphocytes varies somewhat between individual donors, but all samples exhibit saturation of binding above 40 µg per ml of WGA; half-maximal saturation occurs at 6.0 ± 1.4 µg per ml (n = 12). Assuming a M.W. of 25,000 for WGA (7, 8), a maximum of 2.2 ± 1.2 x 10^7 molecules are bound per cell.

Biological Activity of Con A and WGA - The binding of Con A can be compared with its ability to stimulate DNA synthesis (Fig. 1). The mitogenic activity as expressed by thymidine incorporation at 48 hours is a complex function of concentration with maximum stimulation occurring at 80 to 120 µg per ml; higher concentrations are inhibitory. The optimal concentration appears to correspond

to the saturation of at least one class of binding sites. It can be calculated that in this concentration range (80 to 100 μg per ml) the amount of lectin bound corresponds to about $1.8 \pm 0.2 \times 10^7$ molecules per untransformed cell, and $3.7 \pm 0.6 \times 10^7$ molecules per transformed cell (6 experiments). A molecular weight of 10^5 is assumed for Con A (9). At the lowest Con A concentration tested (1 μg per ml), thymidine incorporation is enhanced by the binding of as few as $3.3 \pm 1.1 \times 10^5$ molecules per cell.

In contrast to Con A, WGA does not stimulate DNA synthesis even at concentrations well above those required to saturate the lymphocyte binding sites. WGA does, however, agglutinate lymphocytes under the conditions of the binding assay at lectin concentrations greater than 0.67 μg per ml. No cell agglutination is observed with Con A at any concentration tested unless the temperature is increased to 37°.

Differences in the Changes in Lectin Binding - There are striking differences in the way that Con A- and WGA-binding sites vary during transformation (Fig. 4, Table I). The amount of WGA bound to untransformed lymphocytes is the

TABLE I. Density of binding sites for Con A and WGA on untransformed and transformed human lymphocytes

Calculations are based on data obtained from binding curves at saturation plateaus, 80-100 μg/ml for Con A and 30-80 μg/ml for WGA. The M.W. of Con A was assumed to be 10^5 (9), and for WGA 25,000 (7, 8).

Cells	Concanavalin A		Wheat Germ Agglutinin	
	Molecules per cell	Molecules per μm^2	Molecules per cell	Molecules per μm^2
Untransformed	$1.8 \pm 0.2 \times 10^7$	1.5×10^5	$2.2 \pm 1.2 \times 10^7$	$\simeq 2 \times 10^5$
Transformed	$3.7 \pm 0.6 \times 10^7$	1.5×10^5	$2.2 \pm 1.2 \times 10^7$	$\simeq 7 \times 10^4$

same as that bound to the larger, transformed cells. The density of WGA-binding sites on the cell surface must therefore fall during transformation. In contrast, the number of Con A-binding sites increases during transformation approximately in proportion to the increase in cell surface area. The density of such sites must therefore not change appreciably, and new binding sites must appear in these cells.

DISCUSSION. The heterogeneity of the lymphocyte binding sites for Con A has not been reported previously. Only when many Con A concentrations are examined over a 10^4-fold range with the use of highly radioactive Con A (s.a. 20 μCi per μg) does the complexity of the binding become apparent. Simple Con A saturation

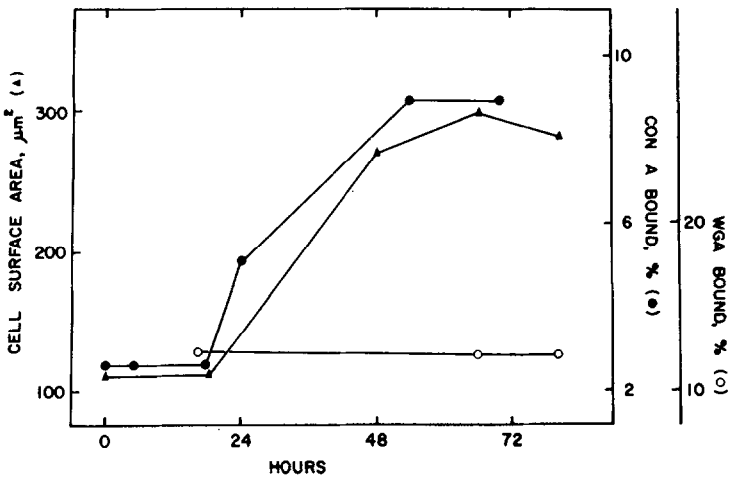

Fig. 4 - Binding of ^{125}I-Con A and ^{125}I-WGA to lymphocytes as a function of surface area during in vitro transformation by Con A. The proportion of lectin added (0 to 1 μg/ml) to the medium that is bound specifically per 10^6 cells is described. The mean surface area per cell was calculated from micrometrically determined mean cell diameters (500 cells per point, 2 experiments).

curves have been observed for certain untransformed and virus-transformed mammalian cells in culture (10-12), as well as for rat and human lymphocytes (13, 14). Comparisons with the present data are difficult, however, since fewer concentrations or narrower ranges were examined in these studies. Large discrepancies exist in the reported binding of Con A to 3T3 and SV3T3 cells. Values for the half-maximal saturation in these cells have varied from 40 to 50 μg per ml (10) to 2 to 5 μg per ml (12). It is possible that these data may refer to different classes of binding sites, depending on the concentration range of Con A selected for study. The present data are in accord with the report of Novogrodsky et al. (14) who found that 1 μg of Con A was bound per 10^6 human lymphocytes at a Con A concentration of 100 μg per ml. Although Con A binding to human lymphocytes was measured at only one Con A concentration, these authors speculated on the existence of heterogeneous binding sites for Con A. The heterogeneity of binding sites is not surprising in view of the complexity of cell-surface carbohydrates and the well-known variability in the affinity of Con A for various saccharides (7, 15-17).

The present data cannot distinguish between the binding of Con A to different sites on distinct cell populations and the binding to different sites on individual cells. Recent data (13) suggest the presence of at least two populations of Con A-binding lymphocytes, one of which is adsorbed to nylon fiber

columns. All of the present experiments were performed with nylon-column-purified lymphocytes, and a similarly complex binding curve is obtained with the presumably homogeneous permanent lymphocyte strain RPMI 6237. Furthermore, gross heterogeneity of binding sites for Con A has been reported for fat cells (5). It is thus likely that a given lymphocyte has different kinds of binding sites on its surface.

The way in which cell surface receptors change during transformation varies greatly among different receptors. During transformation the density of Con A receptors is unchanged, that of WGA falls, and that of insulin (2) increases sharply. A large fall in the density of lectin-binding sites occurs during the rapid reduction of fat cell size observed in starvation (5).

It is of interest to correlate these results with the de novo emergence of insulin receptors which occurs during transformation (2), especially since these receptors are capable of binding the lectins (4). Unfortunately, the number of lectin-binding sites is several orders of magnitude greater than the number of emergent insulin-binding sites (about 10^7 for the lectins, compared with about 10^3 for insulin). The methods used here are not sufficiently sensitive to detect the binding of the lectins to the small number of insulin sites amongst the larger number of unrelated binding sites.

Further studies will be needed to elucidate the complex changes in the properties of the lectin-binding sites in relation to changes in cell growth, function and metabolic state. Important differences in these properties may, for example, exist in neoplastically transformed lymphocytes. Preliminary observations in our laboratory suggest that striking differences exist in the quantity of both of these lectins which can be bound to chronic lymphatic leukemia cells compared with normal cells.

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