Culture Density and Age-Dependent Interactions of 3T3 and SV3T3 Cells with Immobilized and Soluble Lens Culinaris Lectin

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Short term binding of both Balb/c derived 3T3 cells and SV3T3 cells to 2B-Sepharose coated with *Lens culinaris* lectin (LCL) was compared with their LCL-induced agglutinability in relation to culture density and age. The cells were grown for 4 days either to low (LD) or to high density (HD); for certain experiments HD-cells were stimulated (HDS) by a short trypsin treatment and/or by addition of fresh medium. HD-3T3 cells bound somewhat faster to immobilized LCL than LD-3T3 cells, although LD-3T3 cells agglutinated at lower LCL concentrations. In the case of SV3T3 cells, binding was much less pronounced for HD than for LD cells. The agglutinability of HD-SV3T3 cells, however, was greater than that of the other transformed groups. Upon stimulation, binding and agglutinability data of both HD cell lines began to resemble the results obtained with LD-cells. Taken together, the data revealed in all cases an inverse relationship between binding and agglutinability, *i.e.*, high agglutinability was closely correlated with slower binding and vice versa. The results indicate that culture density and age-dependent differences in cell surface architecture can be detected by short term binding to immobilized LCL.

Introduction

The study of cell agglutination by lectins has contributed significantly to our understanding of the dynamic structure of cell membranes. The detection of large variations in lectin-mediated cell-agglutination (for reviews see ref. [1, 2] and lit. therein) made immobilized lectins interesting candidates for affinity separation of intact cells on the basis of cell surface differences (for review see [1, 2]). We have shown that LCL immobilized on beaded agarose of low matrix concentration meets the requirements of specificity, cell viability and high recovery [3, 4].

The present work was undertaken to determine whether cell interaction with immobilized and soluble LCL could reflect qualitative differences in cell surface architecture, as has been described for other lectins [5, 6]. Both 3T3 as well as SV3T3 cells were chosen since they are known to exhibit large variations in agglutinability with ricinus communis agglutinin and Concanavalin A, which are dependent on cell culture conditions and cell density [5, 6]. In this study 3T3 and SV3T3 cells were grown for 4 days to low or high density without medium change and

their interaction with soluble as well as immobilized LCL was determined. Comparison of the agglutinability induced by soluble LCL and of short term binding kinetics to immobilized LCL revealed a specific relationship, indicating that both types of interaction are influenced by similar parameters.

Materials and Methods

Mouse fibroblasts 3T3 and SV3T3, both Balb/c derived, were obtained from Flow Laboratories. The cells were cloned and cultivated as described earlier [4]. Both cell lines were checked routinely and found free of mycoplasms (We thank Dr. Berg for carrying out the assays). LD and HD cultures of 3T3 as well as SV3T3 cells inoculated at different concentrations in order to obtain desired densities were grown for 4 days without medium change in Dulbecco's MEM containing 10% fetal calf serum. After 4 days growth, HD-SV3T3 and HD-3T3 cells reached 7.0×10^5 and 1.1×10^5 cells per cm², respectively; in the case of LD-SV3T3 and LD-3T3 cells 3.5×10^4 and 3×10^4 cells per cm² were obtained. For "stimulation", HD-SV3T3 cells grown for 4 days were fed with fresh medium and allowed to grow for additional 8 h. HD-3T3 cells grown for 6 days (confluent for 2 days) were stimulated for 4 min with 0.001% trypsin (Difco 1:250) in PBS and re-fed with fresh medium; they were then allowed to grow for another 29 h.

For cell suspensions, SV 3 T 3 cells were first detached in a prewarmed (37 °C) solution containing 2 mM EDTA in CMFPBS followed by centrifugation and resuspension in prewarmed 4 mM EDTA in CMFPBS for 10 min. Final resuspension was in the particular solution for the binding or agglutination assay. 3 T 3 cells were washed in prewarmed 4 mM EDTA in CMFPBS two or three times until the cells were removed in the final wash. After centrifugation the cells were resuspended in the assay solution.

The preparation of *Lens culinaris* lectin, its immobilization on large beads of 2 B-Sepharose and the batch technique for reacting cells with immobilized lectin have been reported elsewhere [7]. Sepharose with about 2.5 mg LCL per 1 ml of settled beads was used. The binding reaction was carried out in 6 ml volume containing 0.2 M D-galactose in CMFPBS. For kinetic measurements the number of free cells in the suspension was determined without probing the mechanical stability of the bead-cell complexes. For this purpose a plastic tube mounted with gauze of 100 μm pore size was inserted into the reaction vessel and two 100 μl aliquots were removed from inside the tube as described [4] and counted with a Coulter Counter. The percentage of bound cells was calculated by subtracting the total fraction not bound from the input amount. Each type of experiment to be described was carried out at least 3 times. The relative differences found for the binding of the various cells were highly reproducible, although absolute binding varied somewhat.

The agglutination assay with different amounts of LCL was carried out at room temperature in 1 ml solution of CMFPBS containing 1.5×10^6 cells using a stoppered plastics vial (0.5×2 cm). The vial was turned upside down twice every 5 min. After 30 min two aliquots were transfered to a hemocytometer and were then covered with a coverslide. For scoring agglutination photographs were taken. The number of aggregates per 1000 single cells was calculated for all groups (for this analysis 3 or more cells together were considered an aggregate). The ratio was determined by dividing LCL-induced aggregation by the background aggregation (control cells without lectin).

Size measurements were also taken from enlarged photographs. DNA-histograms were obtained by flow cytophotometry with a computerized cell sorter FACS II (Becton Dickinson; Mountain View Calif.). After fixation of cells with 70% ethanol they were suspended in 0.18 M Tris-HCl, pH 7.5 containing 3 μg/ml DAPI (4,6-diamidino-2-phenylindol-dihydrochloride) as the fluorochrom. Excitation was at 363 nm, analysis was above 396 nm (We thank Dipl. Phys. M. Stöhr for carrying out these measurements).

Results

As with the other lectins [5, 6], major differences were seen in LCL-induced agglutination of cells grown to different densities. The results given in Fig. 1 show that LD-3T3 were more readily agglutinated than HD-3T3 cells, while an inverse relationship was obtained with SV 3 T 3 cells. In this case more LCL was necessary to agglutinate LD cells than HD cells. These data demonstrate that culture density/age-dependent differences in cytoagglutination can be detected by the use of LCL. The differences between non-transformed and transformed cells, however, did not follow the pattern seen with other lectins (for review see [1, 2]).
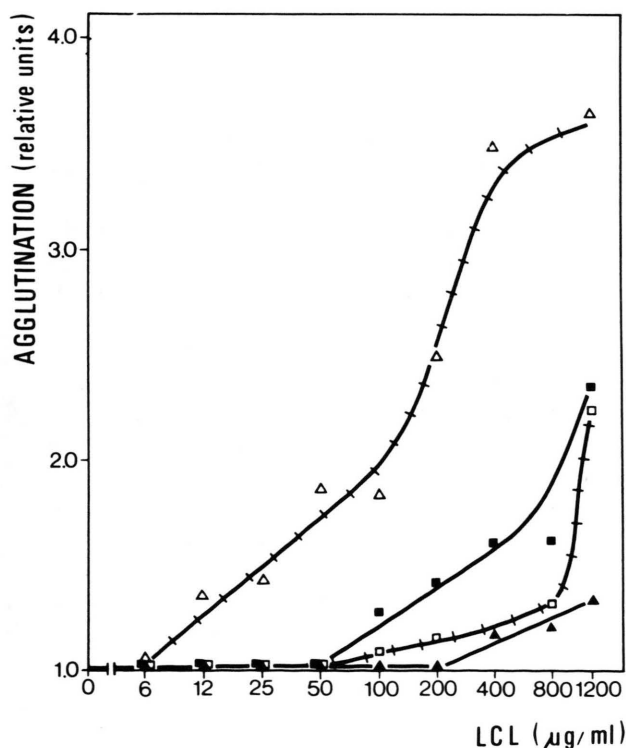

Fig. 1. Cytoagglutination of LD-3T3 (△), HD-3T3 (□), LD-SV 3 T 3 (▲) and HD-SV 3 T 3 cells (■). For details see Materials and Methods.

Fig. 2. (A) Binding of LD-3T3 ($\triangle$) and HD-3T3 cells ($\blacksquare$) to immobilized LCL; 3.6×10^6 cells were reacted with 0.5 ml LCL beads at 34 °C; deviations of the measurements varied between $\pm$ 0.7 and 3%. (B) Binding of LD-SV3T3 ($\triangle$) and HD-SV3T3 cells ($\blacksquare$) to immobilized LCL; 3.2×10^6 cells were reacted with 0.5 ml LCL beads at 34 °C; deviations of the measurements varied between $\pm$ 0.1 and 3.1%.
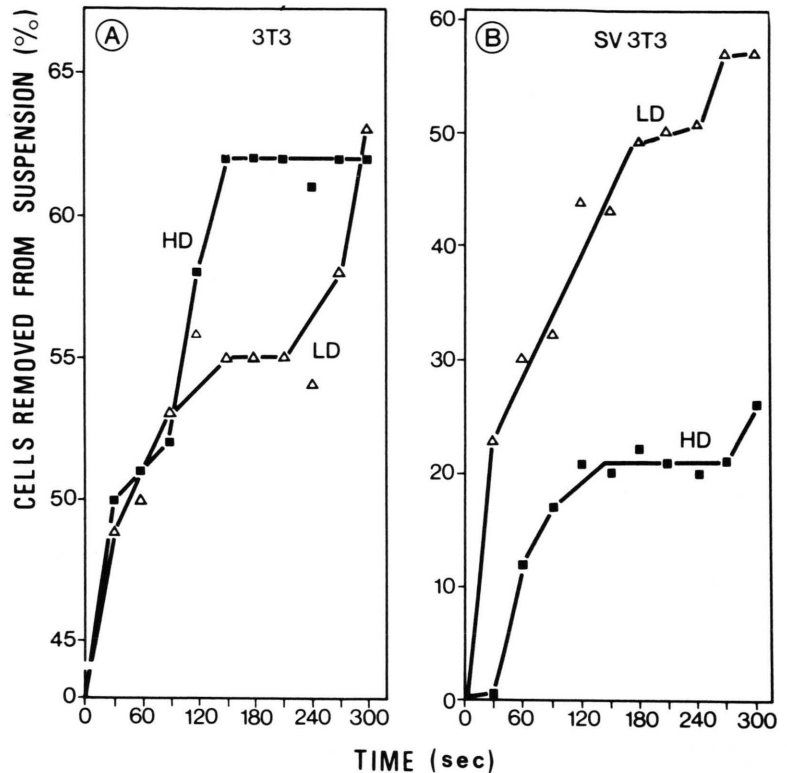
According to current concepts, the binding of intact cells to fixed lectin molecules requires alignment of the cellular receptor sites [8, 9]. The degree of lateral mobility thus seems to be important. Since this is a time-dependent process, differences in cell surface architecture should emerge from short term binding kinetics to immobilized LCL. With 3T3 cells a small but consistent difference in binding kinetics to immobilized LCL was detected (Fig. 2A). More pronounced differences in binding kinetics were found with SV3T3 cells. In the latter case, however, LD-cells bound faster than HD-cells (Fig. 2B). The data from Figs 1 and 2 lead to the preliminary conclusion that cells showing a high agglutinability exhibit slow binding and *vice versa*.

Binding data could be affected by size differences between 3T3 and SV3T3 cells, especially if the behaviour of particles during the binding procedure is considered [4]. The mean diameters expressed in μm $\pm$ SD were as follows: LD-3T3 27.3 $\pm$ 3.4, HD-3T3 25.3$\pm$ 2.1, LD-SV3T3 17.4 $\pm$ 1.3, and HD-SV3T3 15.6 $\pm$ 1.6; *i. e.* LD cells were generally larger than HD cells. With respect to the data shown

in Figs 1 and 2, however, such cell size differences should not play a major role in determining the rate of binding or agglutination.

DNA-histograms seem to rule out that the differences observed may be caused by a different cell cycle staging of the cultures. The original suspensions as well as the non-bound and the released (by the use of 0.2 M methyl-α-D-mannopyranoside; see ref. [7]) fraction gave identical DNA-histograms (data not shown), thus indicating that cells were not preferentially bound in specific phases of the cell cycle.

A major determinant in the degree of binding to immobilized LCL seems to be the age of the culture. SV3T3 cells plated at low or high density 1 day prior to the assay gave only a small although consistent difference in binding (data not shown). As in the case of 4 day old cultures, LD-SV3T3 cells (1×10^5 cells/cm²) bound faster than HD-SV3T3 cells (9.1×10^5 cells/cm²). In the case of one day old 3T3 cultures, however, no difference in binding of LD-cells (0.4×10^5 cells/cm²) *versus* HD-cells (1.5×10^5/cm²) was observed.
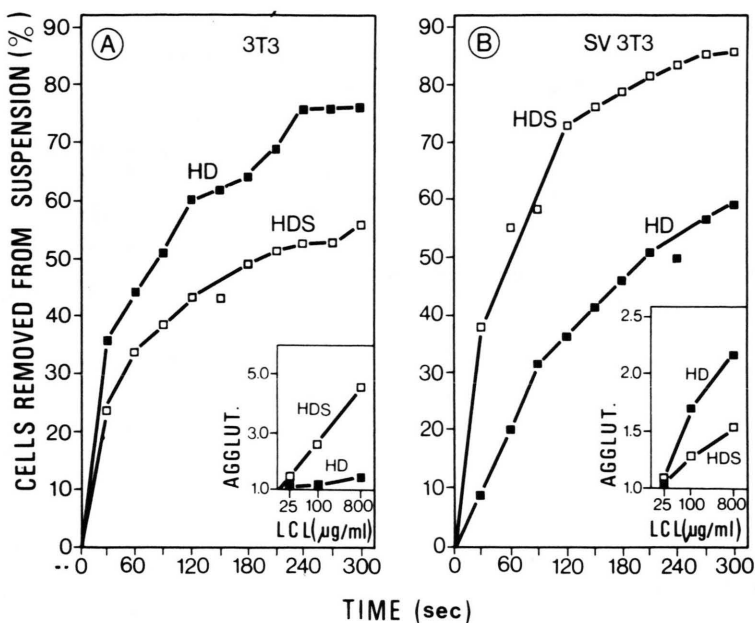
Fig. 3. (A) Binding of HD-3T3 (■) and HDS-3T3 cells (□) to immobilized LCL as compared with the agglutination data (inset); for binding 12.3×10^6 cells were reacted with 0.5 ml LCL beads at 37 °C in the presence of 10 mM methyl-α-D-mannopyranoside; deviations of measurements varied between ± 0.2 and 2.7%. (B) Binding of HD-SV3T3 (■) and HDS-SV3T3 cells (□) to immobilized LCL as compared with the agglutination data (inset); for binding 9.4×10^6 cells were reacted with 0.5 ml LCL beads at room temperature in the presence of CMFPBS; deviations of measurements varied between ± 0.1 and 2.2%.

Besides density and age of cell cultures, the length of incubation with the initially added medium is an important factor. In the case of stimulated HD-3T3 cells, the binding to immobilized LCL decreases when compared with the non-stimulated control; their agglutinability with soluble LCL, however, increases (Fig. 3A). HD-SV3T3 cells, on the other hand, bind faster after stimulation; their agglutinability is diminished (Fig. 3B). In both cell lines binding kinetics as well as agglutinability of HDS-cells are shifted towards values characteristic for LD-cells. In these experiments, again, a high agglutinability was correlated with a slow binding and *vice versa*.

Discussion

It has been possible to demonstrate cell density/age-dependent differences in LCL-induced cytoagglutination as well as in binding of 3T3 and SV3T3 cells to immobilized LCL. Of equal importance is the fact that on stimulation HD cells of both cell lines yield binding kinetics and agglutination data similar to those of the corresponding undisturbed LD-cultures. Kinetics during the first five minutes of binding were examined more closely, since it was repeatedly noted that despite initial differences the endpoint binding after a certain period of time was almost identical. Data by Rutishauser and Sachs [8]

suggest that the degree of lateral mobility of cellular receptor sites might be an important determining factor in the interaction of cells with lectin coated matrices. If this is the case, some parallelism with agglutination data would be expected since receptor mobility also plays an important role in this type of interaction [1, 2]. Such a relationship could in fact be demonstrated for LCL in both forms. Within a given cell line, cells which are more readily agglutinable with soluble LCL bind more slowly to immobilized LCL and *vice versa*. Since high agglutinability, at least for other lectins, is associated to some degree with high mobility of receptor sites, one may speculate that a lower mobility favours binding to immobilized lectin, at least under the conditions employed in this study.

The following considerations support this idea. Lectin-induced agglutination of two cells utilizes mobile receptors on both cells (mobile-mobile). Data by Rutishauser and Sachs [12] indicate that the fixation of one partner cell improves the binding of a native partner cell (immobile-mobile), whereas fixation of both groups abolishes cell-cell binding almost completely (immobile-immobile). The latter situation parallels the failure of fixed cells to bind to immobilized LCL (Kinzel *et al.*, unpublished data) or to Concanavalin A [8]. The kinetically favoured "immobile-mobile" type of interaction occurs in our system, and in fact, the reaction observed happens

extremely rapidly. In the case of the 3 T 3 line 50% of the cells are bound within 30 sec. This is faster than the lectin-mediated agglutination kinetics reported for transformed cells [13]. It can be assumed that the optimum receptor mobilities for binding and agglutination differ completely. Similar relations may also hold for receptor density.

Differential affinity of the receptor sites for lectin depending on cell density [14] may also be important in this system. In addition, a cell type-dependent component may arise during binding simply through a variable ability to stabilize lectin-mediated anchorage on the lectin coated matrix by "unspecific" secondary interactions [4]. Differences in cell cycle staging [15, 16], however, seem not to be involved.

Although lectin-mediated cytoagglutination is the final result of many factors [1, 2], it is significant that within given systems density and age-dependent differences are detectable which can serve as a basis for comparison with binding behaviour to immobilized LCL. We have demonstrated here that despite the possible interfering factors, differences in cell surface architecture can be detected by short term binding to immobilized LCL.

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