CONCANAVALIN-A BINDING BY NORMAL AND TUMOR CELLS

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A method of assessing the binding of concanavalin A by cells of monolayer cultures is described. The method is based on the fact that concanavalin A induces adsorption of red cells on cultures of tumor cells fixed with glutaraldehyde but does not induce adsorption on cultures of normal cells. By using adsorption instead of the classical method of agglutination of living cells, the sensitivity of the reaction can be significantly increased and the possibility of appearance of artifacts arising during work with living cells can be excluded.

An indication of a change in the surface of tumor cells is the increased ability of such cells to be agglutinated by agglutinins of plant origin, notably by concanavalin A (Con A) – a protein from the seeds of Canavalia ensiformis [11]. This protein has two active centers [10] capable of bonding with α-glucopyranoside, α-mannopyranoside, and β-fructofuranoside terminal residues of carbohydrate-containing molecules [9], and it can therefore agglutinate cells with these groups on their surface.

In 1969, Inbar and Sachs [7] first showed that normal and tumor cells differ in their agglutination by Con A. Concentrations of Con A required to agglutinate tumor cells are about 20 times less than are required to agglutinate normal cells. After treatment of normal cells with trypsin, they begin to be agglutinated by Con A in about the same concentrations as tumor cells. This fact has repeatedly been confirmed in lines of normal and tumor cells of different origin, although individual lines of tumor cells are agglutinated like normal cells [3, 8].

A hemadsorption method to assess the binding of Con A by cells of monolayer cultures is suggested in this paper. The method, which will be called the Con A hemadsorption method, is based on the fact that Con A is a powerful agglutinin for guinea pig red cells, and if it is first bound with a fixed monolayer of cells it will therefore cause hemadsorption on the monolayer.

The use of hemadsorption instead of agglutination has several advantages in this case. As will be shown later, it significantly increases the sensitivity of the reaction. It rules out the possibility of a change in the cells during their removal from the monolayer into suspension and subsequent washings [2]. Since the reaction is carried out with fixed cells, there is no possibility of their modification by treatment with the Con A itself. Changes in the surface of cells after treatment with Con A have been deduced from the many effects caused by them, and recently they have been shown by direct experiments [13].

EXPERIMENTAL METHOD

Cultures were grown on coverslips measuring 12 × 12 mm in flasks under antibiotic cover in a mixture containing 45% Eagle's medium, 45% lactalbumin hydrolysate in Hanks's solution, and 10% bovine serum.

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TABLE 1. Minimal Doses of Con A Inducing Positive Con A hemadsorption

Line of cells	ME*	L	M 22	KhIM	S 40	S67	S69	HE†	KhÉTR	KhRO	7l	874
Dose of Con A in μg/ml	>2048	4—8	4—8	8	16	>2048	16	2048	16	8	16	8

* ME – mouse embryonic cells.

† HE – hamster embryonic cells.

Each flask was seeded with 2×10^5 cells in 2 ml medium. The medium was changed once every 2-3 days. The experiments were carried out on cultures aged 4-6 days which had grown to form a compact monolayer.

The cultures were washed with physiological saline in phosphate buffer (SPB), pH 7.4, and fixed with 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 1 h. After fixation the cultures were washed with several changes of SPB for 1.5 h, after which they were treated at room temperature for 1 h with 1 ml of Con A solution in SPB. (The Con A was obtained by the writer's method of specific sorption on Sephadex [1].) At the end of incubation the unbound Con A was removed by washing five times with SPB, after which the culture was treated for 1 h with a 0.5% suspension of guinea pig red cells in SPB. (The red cells were obtained by cardiac puncture, collected in Alsever's solution, and washed three times with SPB before the experiment.) The unbound red cells were removed by washing in SPB, and the coverslips were dried and the degree of hemadsorption estimated visually. The reaction was considered positive if clearly visible, uniform adsorption of the red cells had occured on the surface of the coverslip.

As a control of the specificity of the reaction, instead of Con A the test was carried out with a solution of Con A in 10^{-2} M α-methylglucopyranoside, a specific inhibitor of Con A [5]. In that case the red cell suspension also contained inhibitor in a concentration of 10^{-2} M. When the inhibitor was used the reaction was negative regardless of the doses of Con A or the lines of cells tested.

It must be emphasized that a compact layer of cells is essential for the test to be successful. This is because the cultures are grown in medium containing 10% serum. Under these circumstances some of the serum is irreversibly adsorbed on the coverslip [12], and since some components of the serum are able to bind with Con A [6], this causes hemadsorption on the coverslip even if no cells are present, making the results difficult to interpret.

The following cell lines were used in the work.

1. Mouse: cultures obtained from sarcomas induced in the animals by implantation of polyvinyl chloride disks (lines KhIM, S 50, S 67, S 69); embryonic cells transformed by virus SV 40 (line M 22); embryonic cells transformed by methylcholanthrene (line L [4]). All these lines except M 22 were oncogenic for animals, and for the lines obtained from the mouse "plastic" sarcomas, LD_{50} was less than 100 cells if injected subcutaneously into adult syngeneic unirradiated mice.

2. Hamster: Spontaneously transformed embryonic cells (line KhÉTR); cultures obtained from tumors induced by Rous virus (line KhRO), virus SV 40 (line 7l), and polyoma virus (line 874).

Secondary cultures of embryonic fibroblast-like cells of mice and hamsters were used as the normal cells. At least three experiments were carried out with each line.

EXPERIMENTAL RESULTS

Minimal doses of Con A giving a positive Con A hemadsorption reaction for different lines are given in Table 1. All tumor cells except S 67 gave a positive reaction with doses of Con A of not more than 16 μg/ml, whereas the reaction of normal cells was negative even with Con A in a dose of 2048 μg/ml (the highest concentration used).

Of all the lines studied, only S 67 cells, which are highly oncogenic for mice, behaved like normal cells in the reaction. The Con A hemadsorption method can thus distinguish readily between normal and tumor cells in a culture although, just like the agglutination reaction, some neoplastic lines behave in the hemadsorption reaction like normal lines.

The effect of trypsin on Con A hemadsorption on normal cells also was studied. Treatment of normal cells with trypsin is known to increase their agglutination induced by Con A [7]. A culture on a coverslip

was treated with 0.1% crystalline trypsin (Spofa, Czechslovakia) solution in SPB at room temperature and then quickly washed with a large volume of SPB and fixed. The control culture was treated with a solution containing 0.1% trypsin and 0.2% trypsin inhibitor from soy seeds (Sigma, USA). The minimal dose of Con A producing positive Con A hemadsorption for cultures treated with trypsin was 4 μg/ml, whereas in the control series the dose was greater than the highest concentration used (2048 μg/ml).

The results obtained by this method thus agree with data in the literature on the agglutination reaction. However, the method described above is much more sensitive, for the doses of Con A which give positive hemadsorption on tumor cells are at least 200 times smaller than the corresponding doses for normal cells. The corresponding doses for the agglutination reaction differ by about 20 times.

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